

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Engelhardt, <i>et al.</i>)	
)	

Serial No.:	08/479,997)	Group Art Unit: 1631
)	

Filed:	June 7, 1995)	Ex'r: Ardin H. Marschel, Ph.D.
)	

For:	OLIGO- OR POLYNUCLEOTIDES)	
	COMPRISING PHOSPHATE MOIETY)	
	LABELED NUCLEOTIDES)	
	(As Previously Amended))	

South Portland, Maine 04106

Commissioner for Patents
Washington, D.C. 20231

SUPPLEMENTAL DECLARATION OF DR. ALEX A. WALDROP, III

I, Alex A. Waldrop, III, hereby declare as follows:

1. I am the same Dr. Alexander A. Waldrop, III who submitted a Declaration in the above-identified application in June 2002. My professional background, education, training and experience are as described in my *curriculum vitae* (cv) attached as Exhibit 1 to my June 2002 Declaration. A recent cv is attached, however, as Exhibit A to this Supplemental Declaration.

2. Enzo Life Sciences, Inc. has requested that I review as its scientific consultant significant portions of the most recent prosecution history of United States Patent Application Serial No. 08/479,997, filed on June 7, 1995 ("the '997

Enz-5(D6)(C2)

application") in the name of Dean L. Engelhardt, *et al.* as inventors. The title of the Engelhardt application is "Oligo- or Polynucleotides Comprising Phosphate Moiety Labeled Nucleotides." Included for this particular review were the following documents:

- two Office Actions dated July 14, 2004 and November 26, 2003;
- four documents cited in the November 26, 2003 Office Action:
 - Lehninger, Biochemistry, Worth Publishers, Inc., New York, NY, 1970, pages 638-639;
 - Hartman et al., "Methacrylate Polymerization by AzoRNA: Potential Usefulness for Chromosomal Localization of Genes," Biopolymers 20:2635-2648 (1981);
 - Dunn et al., "A Novel Method to Map Transcripts: Evidence for Homology between an Adenovirus mRNA and Discrete Multiple Regions of the Viral Genome," Cell 12:23-36 (1977); and
 - Hung et al., U.S. Patent No. 4,224,408;
- Applicants' April 23, 2004 Amendment Under 37 C.F.R. §1.115 (In Response To the November 26, 2003 Office Action);¹
- A set of claims that are being submitted in response to the July 14, 2004 Office Action;^{2,3}

¹ I understand that the previously pending claims, 826-1227, were submitted in Applicants' April 23, 2004 Amendment.

² Copy attached as Exhibit B.

³ The set includes claims 826, 828-832, 835-847, 849-856, 858-862, 865-878, 880-888, 890-894, 897-909, 911-921, 923-927, 930-943, 945-956, 958-961, 964-976, 978-988, 990-993, 996-1009, 1011-1022, 1024-1027, 1030-1042, 1044-1054, 1056-1059, 1062-1075, 1077-1088, 1090-1094, 1097-1112, 1114-1121, 1123-1127, 1130-1146, 1148-1156, 1158-1162, 1165-1177, 1179-1191, 1193-1197, 1200-1213 and 1215-1227. Twelve claims (826, 856, 888, 921, 956, 988, 1022, 1054, 1088, 1121, 1156 and 1191) are independent and each of these has been amended.

- the patent specification filed on June 7, 1995 (but claiming priority to June 23, 1982) [hereinafter "the '997 specification"]; and
- three legal cases:
 - *In re Grasselli* (PTO Board of Appeals & Interferences, 1983);
 - *Ex parte Pearson* (PTO Board of Appeals & Interferences, 1985); and
 - *Ex parte Parks* (PTO Board of Appeals & Interferences, 1993).

I am being compensated for my review and for making this Supplemental Declaration.

3. Based upon my review of the claims being submitted to the U.S. Patent Office (Exhibit B), I understand that the invention in the '997 application is directed to oligo- or polydeoxynucleotides (or oligo- or polynucleotides) comprising modified nucleotides or nucleotide analogs. Such claimed oligo- or polynucleotides are useful as hybridization probes for detecting nucleic acids of interest. I understand that in each of the twelve independent claims, a detectable non-polypeptide, *non-nucleotidyl*, non-radioactive label moiety (termed "Sig") is attached to the phosphate moiety of at least one modified nucleotide or modified nucleotide analog in the oligo- or polynucleotide.⁴ In four of the independent claims (956, 988, 1022 and 1054), the Sig component is further defined as being biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component,

⁴ Claim 826, which is representative of the twelve independent claims, recites:

An oligo- or polydeoxyribonucleotide . . . comprising at least one modified nucleotide or modified nucleotide analog having the formula Sig--PM--SM--BASE, wherein PM is a phosphate moiety, SM is a furanosyl moiety and BASE is a base moiety or a base analog . . . wherein said analog can be attached or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, wherein said PM is attached to SM, said BASE is attached to SM, said Sig is covalently attached to PM directly or through a chemical linkage, and wherein said Sig comprises a non-polypeptide, non-nucleotidyl non-radioactive label moiety comprising at least three carbon atoms and which Sig can be directly or indirectly detected when attached to PM . . .

a fluorescent component, a chemiluminescent component, a chromogenic component, or a combination of any of the foregoing.

4. I have read the July 14, 2004 Office Action in which the Patent Examiner rejected all of the claims, 826-1227, as failing to comply with the written description requirement because of a "'non-nucleotidyl' limitation directed to the Sig moiety species."⁵

5. As Enzo's scientific consultant, I am making this Supplemental Declaration in support of the subject matter claimed in the '997 application, and in particular, to the definition recited in the claims that the non-radioactive Sig component is "non-nucleotidyl." I have been informed that my Supplemental Declaration will be submitted to the U.S. Patent Office as part of a response to the July 14, 2004 Office Action.

6. As set forth in my previously submitted cv, I am a chemist with substantial experience and background in nucleic acid chemistry. My knowledge, background, training and experience in nucleic acid chemistry encompasses nucleic acid modifications, including labeling nucleic acids for use in hybridization and detection

⁵ The full text of the Examiner's comments is set forth on page 2 in the July 14, 2004 Office Action and provides:

The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

NEW MATTER has been newly added to the claims due to the added "non-nucleotidyl" limitation directed to Sig moiety species. Applicants have not pointed to written basis as filed for this limitation nor has written basis as filed been found via consideration of the entirety of the instant disclosure as filed. It is acknowledged that numerous types of Sig moieties have been exemplified as filed, however, a clear delineation of such moieties to support the "non-nucleotidyl" limitation has not been found. All independent claims contain this NEW MATTER limitation. Claims which depend from independent claims either directly or indirectly also contain this NEW MATTER due to their dependence. This rejection is necessitated by amendment.

assays. I am familiar with several nucleic acid detection formats and with nucleic acid probe technology in general. My professional and academic career involves extensive research exploring the modifications and labeling of nucleic acids for use as probes in hybridization and detection assays. One of my more recent areas of research is chemiluminescence and assays using chemiluminescent reagents to detect a wide variety of substances including nucleic acids and other biomolecules.

7. Based upon my education, training, background and experience, I believe that at the time the first parent application of the pending '997 application was filed, in June 1982, the relevant art to the claimed modified oligo- or polynucleotides would have included many if not most of the following areas: modifications of nucleic acids, nucleic acid synthesis and labeling, nucleic acid hybridization, formatting and detection. I consider myself to possess the level of skill, knowledge, training and experience of at least a person skilled in the art to which the present modified oligo- or polynucleotide invention pertains.

8. I understand that a patent specification describes the subject matter of a claim, if the specification conveys, with reasonable clarity to a person skilled in the art, that the inventors were in possession of the subject matter recited in that claim. I also understand that to satisfy the written description requirement, the inventors do not have to utilize any particular form of disclosure to describe the subject matter of the claim under consideration. For instance, the description of the invention being claimed may be found in the working examples, in a more general description of the invention, or even in a combination of the examples and the general description.

9. As a person skilled in the art, it is my opinion and conclusion that the '997 specification reasonably conveys that at the time their application was filed in June 1982, Applicants were in possession of the claimed invention directed to the non-

nucleotidyl Sig component. For reasons which are given below, I believe that the '997 specification reasonably conveys that the Sig component is non-nucleotidyl. Furthermore, as explained below, I believe that any inference from the '997 specification and pending claims that the Sig component is or could be nucleotidyl in nature, or that Sig comprises a nucleotide, would be erroneous and unreasonable.

NONE OF THE EXAMPLES FOR THE SIG LABEL MOIETY ARE NUCLEOTIDYL OR A NUCLEOTIDE

10. At the outset I find it significant that the '997 specification discloses several examples for the Sig component -- all of which show that Sig is neither a nucleotide nor that it is nucleotidyl in its nature. Beginning on page 96, last paragraph, and continuing through the first paragraph on page 97, the '997 specification discloses:

The Sig moiety employed in the make-up of the special nucleotides of this invention could comprise an ***enzyme or enzymic material***, such as ***alkaline phosphatase, glucose oxidase, horseradish peroxidase*** or ***ribonuclease***. The Sig moiety could also contain a ***fluorescing component***, such as ***fluorescein*** or ***rhodamine*** or ***dansyl***. If desired, the Sig moiety could include a ***magnetic component*** associated or attached thereto, such as a ***magnetic oxide*** or ***magnetic iron oxide***, which would make the nucleotide or polynucleotide containing such a magnetic-containing Sig moiety detectable by magnetic means. The Sig moiety might also include an ***electron dense component***, such as ***ferritin***, so as to be available by observation. The Sig moiety could also include a ***radioactive isotope component***, such as ***radioactive cobalt***, making the resulting nucleotide observable by radiation detecting means. The Sig moiety could also include a ***hapten component*** or per se be capable of complexing with an antibody specific thereto. Most usefully, the Sig moiety is a ***polysaccharide or oligosaccharide or monosaccharide***, which is capable of complexing with or being attached to a sugar or polysaccharide binding protein, such as a lectin, e.g. Concanavilin A. The Sig component or moiety of the special nucleotides in accordance with this invention could also include a ***chemiluminescent component***. [emphasis added]

Of the approximately twenty different examples for Sig that have been bolded in the passage above, none comprises a nucleotide or the three nucleotidyl subunits (sugar, phosphate and base) necessary to build the nucleotide structure.⁶

THE SIG LABEL MOIETY IS ATTACHED TO THE PHOSPHATE MOIETY OF A NUCLEOTIDE

11. I also find it significant that the '997 specification and the pending claims describe the claimed Sig component in terms of its attachment to the phosphate moiety of a nucleotide.

i) Page 94, last paragraph:

Still further, nucleotides in accordance with the practices of this invention include the nucleotides having the formula,

Sig

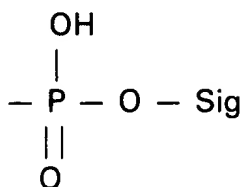
|

P — S — B

wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety. In these special nucleotides the P moiety is attached to the 3' and/or the 5' position of the S moiety when the nucleotide is deoxyribonucleotide and at the 2', 3' and/or the 5' position when the nucleotide is a ribonucleotide. The base B is either a purine or a pyrimidine and the B moiety is attached from the N1 or the N9 position to the 1' position of the sugar moiety when said B moiety is a pyrimidine or a purine, respectively. ***The Sig chemical moiety is covalently attached to the phosphoric acid P moiety*** via the chemical linkage

⁶ Lehninger, cited in the November 23, 2003 Office Action provides the following description for a nucleotide on page 55, last paragraph:

The recurring structural units of all nucleic acids are eight different *nucleotides*; four kinds of nucleotides are the building blocks of DNA, and four others are the structural units of RNA. Each nucleotide in turn contains three smaller units: [1] a nitrogenous organic base, [2] a 5-carbon sugar, and [3] phosphoric acid . . .



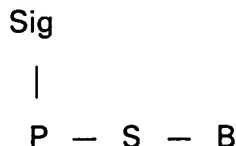
said Sig, when attached to said P moiety being capable of signalling itself or making itself self-detecting or its presence known and desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid and when so incorporated therein is still self-detecting. [emphasis added]

ii) Page 97, first full paragraph:

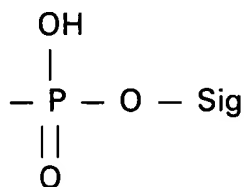
As indicated *in accordance with the practices of this invention*, the **Sig component** could comprise any chemical moiety which is **attachable** either directly or through a chemical linkage or linker arm **to the nucleotide**, such as **to the [phosphate] P component (PM)** thereof. [emphasis added]

iii) Originally filed claim 141:

141. A nucleotide having the general formula



wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety, the phosphoric acid moiety being attached to the 3' and/or the 5' position of the sugar moiety when said nucleotide is deoxyribonucleotide and at the 2', 3' and/or 5' position when said nucleotide is a ribonucleotide, said base B being a purine or pyrimidine, said base B moiety being attached from the N1 or the N9 position to the 1' position of the sugar moiety when said base B is a pyrimidine or a purine, respectively, and wherein **Sig is a chemical moiety is covalently attached to the phosphoric acid moiety** via the chemical linkage



said Sig, when attached to said phosphoric acid moiety P being capable of signalling itself or making itself self-detecting or its presence known. [emphasis added]

12. In the case of ii), the above-quoted passage plainly omits from the definition of the Sig component all practices not "in accordance with the practices of this invention." One such practice readily recognized by a person of skill in the art as not being in accordance with the invention is the alleged embodiment where the Sig component comprises a nucleotide. The passage in i) of Paragraph 11 above makes it clear that *Sig is covalently attached to the phosphoric acid P moiety*. Apart from the inclusion of Sig, the structure given in i) of Paragraph 11 is that of a nucleotide with its three subunits. If Sig were to comprise a nucleotide, then Sig would necessarily comprise the three subunits for a nucleotide. There is no description in the '997 specification, including the passage quoted in Paragraph 10 above, that shows Sig to comprise the three subunits required for the nucleotide structure. As explained in Paragraph 10, none of the twenty different examples of Sig that are bolded in the quoted passage comprise a nucleotide or the three subunits of a nucleotide.

BECAUSE THE '997 SPECIFICATION REFERS IN SEVERAL INSTANCES TO A "SIG-CONTAINING NUCLEOTIDE," OR TO "THE RESULTING NUCLEOTIDE," SIG CANNOT LOGICALLY BE NUCLEOTIDYL OR A NUCLEOTIDE ITSELF

13. Furthermore, it is clear from the passage quoted in Paragraph 11, ii) that the "Sig component" of the nucleotide is not itself a nucleotide. If the Sig component comprised a nucleotide, the reference in the above quoted passages to Sig as a *component* of "the nucleotide" would make no sense. Indeed, the '997 specification is replete with evidence that the Sig component cannot comprise a nucleotide. In several instances, the '997 specification refers to "Sig-containing

nucleotide" or to "the resulting nucleotide" (and the like). Some examples are provided below.

i) Page 95, lines 2-13:

The Sig chemical moiety is covalently attached to the phosphoric acid P moiety (PM) via the [phosphate] chemical linkage . . . said Sig, when attached to said P moiety (PM) being capable of signalling itself or making itself self-detecting or its presence known and desirably **the nucleotide** is capable of being incorporated into a double-stranded polynucleotide . . . [emphasis added]

ii) Page 96, lines 12-20

The chemical moiety Sig so attached to the nucleotide P-S-B (PM-SM-BASE) is capable of rendering or making the **resulting nucleotide**, now comprising P-S-B (PM-SM-BASE) with the Sig moiety being attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide. . . [emphasis added]

iii) Page 96, lines 22-28

The Sig moiety desirably should not interfere with the capability of **the nucleotide** to form a double-stranded polynucleotide containing the **special Sig-containing nucleotide** in accordance with this invention and, when so incorporated therein, the **Sig-containing nucleotide** is capable of detection, localization or observation. [emphasis added]

iv) Page 99, lines 6-10

As indicated, such probes may contain one or more of the **special Sig-containing nucleotides** in accordance with this invention, preferably at least about **one special nucleotide** per 5-10 of the nucleotides in the probe. [emphasis added]

14. The just-quoted passages refer to the Sig-containing nucleotide as "the nucleotide," "the special Sig-containing nucleotide," "one special nucleotide," and "the resulting nucleotide," If the Sig component itself were a nucleotide, these passages would make little if any sense. There would be no "resulting nucleotide," rather, there would be a "resulting *dinucleotide*." Further, there would be no "one special nucleotide," but rather "a special *dinucleotide*." To illustrate this point more

clearly, let us presume that the Sig component of "the nucleotide" were itself a nucleotide. From this presumption, it follows that every reference in the specification to "a Sig-containing nucleotide" or the like necessarily refers to a "nucleotide-containing nucleotide," or to an oligo- or polynucleotide. This leads to the anomalous result that the modified nucleotide of the invention is really not a nucleotide at all but, rather, is a *polynucleotide* or a dinucleotide of sorts. This defies both the dictionary⁷ and commonsense. Further, it contravenes the '997 specification which, as shown above, makes clear that "Sig-containing nucleotide" refers to a single nucleotide, and not a polynucleotide or a dinucleotide. The '997 specification never suggests, explicitly or implicitly, that the "Sig-containing nucleotide" could be a polynucleotide or dinucleotide. Indeed, as shown above, the specification teaches that the "Sig-containing nucleotide" is "*one* special nucleotide". Accordingly, one skilled in the art would recognize that the "Sig-containing nucleotide" of the claimed invention refers to a single nucleotide and thus that Sig cannot logically be nucleotidyl or a nucleotide itself.

15. In summary, it is my opinion as a person skilled in the art to which the '997 application and invention pertains, that the recited Sig component cannot be nucleotidyl or a nucleotide. As explained above, the various examples of Sig that are described in the '997 specification do not fit the definition of a nucleotide because the three nucleotidyl subunits necessary to constitute a nucleotide are altogether lacking in the Sig descriptions. Furthermore, as I explained above, to assert that the claimed Sig component is a nucleotide would lead to the erroneous and unreasonable conclusion that Sig can be at the same time both a component of

⁷ According to the American Heritage[®] Dictionary of the English Language (4th Ed. 2000), "nucleotide" is defined as "[a]ny of various compounds consisting of a nucleoside combined with a phosphate group and forming the basic constituent of DNA and RNA." The dictionary defines "oligonucleotide" as "a short polymer of *two to twenty nucleotides*." Finally, "polynucleotide" is defined as a "polymeric compound, usually DNA or RNA, consisting of a *number of nucleotides*."

a nucleotide (much like the sugar, phosphate and base moieties), and a nucleotide itself. A reading of the '997 specification clearly shows that Sig can only be a component of a nucleotide, and that it is not nucleotidyl or a nucleotide. Moreover, as explained earlier, Sig cannot be a nucleotide because it would force a person skilled in the art to define wrongly in several instances the word "nucleotide" in the '997 specification to mean or to refer to "a polynucleotide," "an oligonucleotide," or to "a dinucleotide."

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Sept. 3, 2004
Date

Dr. Alex A. Waldrop, III
Dr. Alex A. Waldrop, III

* * * * *

FinalDecl.9.2.04(8PM)

Curriculum Vitae of Alex A. Waldrop, III

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EDUCATION

Ph.D. (Biophysics) 1977, Johns Hopkins University, Baltimore, Maryland,
Thesis Advisor: Dr. Michael Beer,
Thesis Title: "Chemical Studies "Chemical Studies of
bis(Pyridine)osmate(VI) Esters and the Mercury
Enhancement of Osmium Labelling of Polynucleotides"
Dissertation Abstracts International 38 (11-B):5354+
(194 pp.) (1978);
B.S. (Chemistry) 1970, Magna cum Laude, University of Virginia.

HONORS

Echols Scholar, Phi Eta Sigma, Hugh Miller Spencer Scholarship in
Chemistry, 1970.

PROFESSIONAL MEMBERSHIPS

Alpha Chi Sigma, Sigma Xi, AAAS, AACC, American Chemical Society.

EXPERIENCE

Founder and Principal Scientist, Started Company at Center for
Environmental Enterprise (CEE), 2000 to present. Further characterized
9-Acridinecarbonylimidazole (AcriGlow 301) and its reaction with
peroxide in various buffers and solvents. Examined ways of removing
peroxide impurities from solvents, detergent and polymer solutions.
Tested screening assay for detecting pollutants in environmental water
samples. Served as consultant for Brims Ness, Capricorn Products, Inc.,
Maine Standards, and Enzo Biochem, Inc.

Visiting Scientist, Maine Medical Center Research Institute, 1994 to
2000. Synthesized and characterized modified acridancarboxylic acid
ester. Demonstrated substrate activity with HRPO. Invented and
characterized activated 9-acridinecarboxylic acid derivatives.
Demonstrated high sensitivity assay of glucose oxidase and alkaline
phosphatase. HPLC of acridine derivatives. HPLC of synthetic
oligonucleotides.

Research Scientist, IDEXX Laboratories, Inc., 1992 - 1993.
Optimization of HRPO assay systems.

Staff Scientist, Gen-Probe, Inc., 1985 - 1992. Synthesized and designed
acridinium esters. Helped design linker arms, optimize detection of

acridinium esters, stabilize acridinium esters, improve elution of nucleic acids from solid supports. Characterized acridinium esters by HPLC, UV and chemiluminescence.

Research Associate, Department of Microbiology, University of Virginia, 1982 - 1985. Developed new DNA sequencing method similar to Sanger approach, but which leaves functional 3' ends, which can be ligated to produce a set of deletion mutants or can be extended under conditions forcing misincorporation to generate a set of point mutations. Synthesized series of 5'-thymidine triphosphate derivatives containing a 3'-phosphate mono-, di-, or triester group. Showed that these analogs were not substrates for T4 or Klenow DNA polymerase. Developed simple, rapid gel filtration method for purifying and desalting nucleotides. Synthesized an analog of dUTP containing an EDTA group and showed that it can be enzymatically incorporated into DNA.

Assistant Professor, Department of Chemistry, University of Virginia, 1980-1982. Prepared nucleotide derivatives of tubercidin. Characterized allylamine derivatives. Taught biophysical chemistry.

Postdoctoral Research Fellow, Department of Molecular Biophysics and Biochemistry (laboratory of Dr. David C. Ward), Yale University, 1977-1980. Synthesized modified pyrimidines to incorporate in vitro into nucleic acids, using reactions between heavy metals and nucleic acid components. Developed nucleotide analogs used for gene detection in situ. Biotinyl nucleotides now selling commercially.

Predoctoral Fellow, Department of Biophysics (laboratory of Dr. Michael Beer), Johns Hopkins University, Baltimore, Maryland, 1970-1977. Developed multiple heavy atom stains for electron microscopy of nucleic acids.

ACHIEVEMENTS Co-inventor of non-radioactively-labeled nucleotides, including biotinyl nucleotides (U.S. Patents Nos. 4,711,955; 5,328,824; 5,449,767; and 5,476,928). Co-inventor of activated 9-acridinecarboxylic acid chemiluminescent system. Experienced in chemistry of nucleic acids and proteins, especially the synthetic chemistry of nucleotides, peptides, and their oligomers, and in the chemistry of mercury, osmium, and palladium; familiar with NMR, UV-Visible, IR, and fluorescent spectroscopic techniques, and with TLC, HPLC, gel filtration, and ion exchange chromatographic procedures; experienced in the use of DNA polymerases and nucleases. Experienced in detection systems for nucleic acids, especially chemiluminescence. Experienced in chemistry of acridine and acridinium compounds. Experienced with several ELISA enzymes,

including horseradish peroxidase (HRPO), alkaline phosphatase, glucose oxidase, and β -galactosidase.

Publications

- (1) Richardson, F.S., Shillady, D.D., Waldrop, A.A.; A Theoretical Study of Cis-Trans Photoisomerization in the Bis(Glycinato) Platinum(II) Complex, Inorganica Chimica Acta, 5, 279-289 (1971).
- (2) Waldrop, A.A., Beer, M., Marzilli, L.G.; Osmium-labeled Polynucleotides. Incorporation of Additional Heavy Atoms (Mercury) via Ligand Substitution Reactions, Journal of Inorganic Biochemistry, 10, 225-234 (1979).
- (3) Langer P.R., Waldrop, A.A., and Ward, D.C.; Enzymatic Synthesis of Polynucleotides Containing Biotin: Novel Nucleic Acid Affinity Probes, Proc. Natl. Acad. Sci. U.S.A., 78, 6633-6637 (1981).
- (4) Hammond, Philip W.; Wiese, Wendy A.; Waldrop, Alex A., III; Nelson, Norman C.; Arnold, Lyle J., Jr.; Nucleophilic Addition to the 9 Position Of 9-Phenylcarboxylate-10-Methylacridinium Protects Against Hydrolysis of the Ester, J. Biolumin. Chemilumin. 6(1), 35-43, (1991).
- (5) Waldrop, Alex A., III; Fellers, Jonathan; Vary, Calvin P. H.; Chemiluminescent Determination of Hydrogen Peroxide with 9-Acridinecarbonylimidazole and Use in Measurement of Glucose Oxidase and Alkaline Phosphatase Activity, Luminescence 15(3), 168-182, (2000).

Patents and Patent Applications

- (1) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Modified Nucleotides and Methods of Preparing and Using Same, U.S. Patent 4,711,955 (December 8, 1987). (European Pat. Appl. EP 63879 A2)
- (2) Arnold, Lyle J., Waldrop, Alex A., III, Hammond, Philip W.; Protected Chemiluminescent Labels, U. S. Patent # 4,950,613 (Aug. 21, 1990). (European Pat. Appl. EP 330433 A2).
- (3) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Methods of Using Labeled Nucleotides. U.S. Patent #5,328,824 (July 12, 1994).
- (4) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Modified Polynucleotides and Methods of Preparing Same. U.S Patent #5,449,767 (Sept.12, 1995).
- (5) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Modified Nucleotides and Polynucleotides and Complexes Form Therefrom. U.S Patent #5,476,928 (Dec.19, 1995).

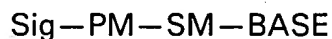
- (6) Arnold, Lyle, J., Jr.; Nelson, Norman C.; Reynolds, Mark A.; Waldrop, Alex A., III; Polycationic Supports and Nucleic Acid Purification, Separation and Hybridization. U. S. Patent #5,599,667 (Feb 4, 1997). (European Pat. Appl. EP 281390 A2).
- (7) Waldrop, Alex A., III and Vary, C.P.H., Peroxide-Based Chemiluminescent Assays and Chemiluminescent Compounds Used Therein. Patent pending (Submitted 1997 as Provisional Patent Application).

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EXHIBIT B TO DECLARATION OF DR. ALEX A. WALDROP, III

Claims 1-825 (Canceled)

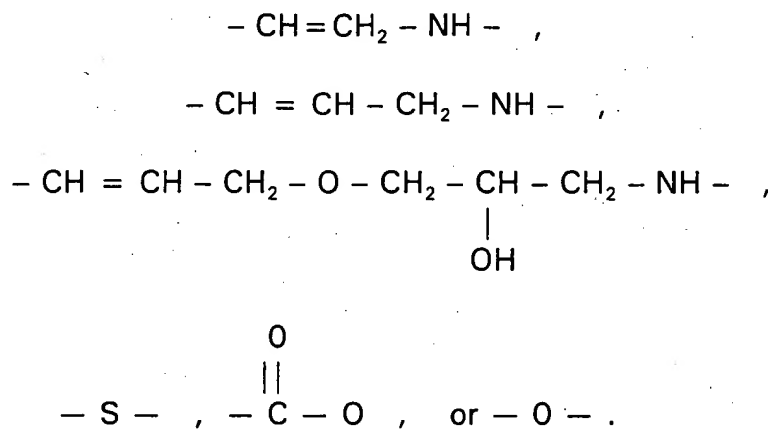
826. (Currently Amended) An oligo- or polydeoxyribonucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide or modified nucleotide analog having the formula



wherein PM is a phosphate moiety, SM is a furanosyl moiety and BASE is a base moiety or a base analog comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, wherein said PM is attached to SM, said BASE is attached to SM, said Sig is covalently attached to PM directly or through a chemical linkage, and wherein said Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety comprising at least three carbon atoms and which Sig can be directly or indirectly detected when attached to PM or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polydeoxyribonucleotide or when said oligo- or polydeoxyribonucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof.

827. (Canceled)

1169. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said polypeptide chemical linkage further comprises any of the moieties:



1170. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said polypeptide chemical linkage further comprises a glycosidic linkage moiety.

1171. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said PM comprises a monophosphate, a diphosphate or a triphosphate.

1172. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oxygen.

1173. (Currently Amended) The oligo- or polynucleotide of claim 1156, wherein said Sig moiety is attached via said polypeptide chemical linkage to the furanosyl moiety PM of a terminal nucleotide in said oligo- or polynucleotide.

1174. (Previously Added) The oligo- or polynucleotide of claim 1173, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

1175. (Previously Added) The oligo- or polynucleotide of claim 1173, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 2' position thereof.

1176. (Previously Added) The oligo- or polynucleotide of claim 1174, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 3' position thereof.

1177. (Previously Added) The oligo- or polynucleotide of claim 1175, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 3' position thereof.

1178. (Canceled)

1179. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

1180. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

1181. (Previously Added) The oligo- or polynucleotide of claim 1180, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

1182. (Previously Added) The oligo- or polynucleotide of claim 1180, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

1183. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs or a combination of any of the foregoing.

1184. (Previously Added) The oligo- or polynucleotide of claim 1183, wherein said adenosine analogs comprise tubercidin and toyocamycin.

1185. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

1186. (Currently Amended) The oligo- or polynucleotide of claim ~~1185~~ 1156, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

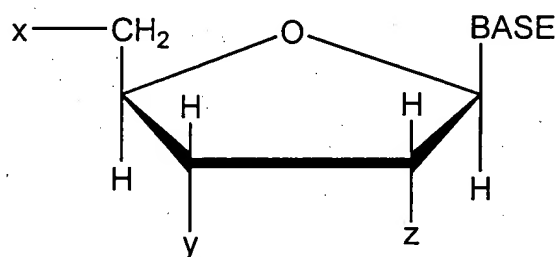
1187. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

1188. (Currently Amended) The oligo- or polynucleotide of claim ~~1187~~ 1156, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one ~~deoxyribonucleotide~~ deoxyribonucleotide.

1189. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said polypeptide chemical linkage comprises polylysine.

1190. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said polypeptide chemical linkage comprises avidin, streptavidin or anti-hapten immunoglobulin.

1191. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide or modified nucleotide having the structural formula:



wherein BASE is a base moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, and wherein said BASE is attached to the 1' position of the furanosyl ring from the N1 position when said BASE is a pyrimidine or a pyrimidine analog, or from the N9 position when said BASE is a purine, a purine analog, a deazapurine or a deazapurine analog;

wherein x comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein y comprise H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein z comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate; and

wherein Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label comprising at least three carbon atoms, which Sig is covalently attached through a chemical linkage to at least one phosphate comprising x, y, z, or a combination thereof, said chemical linkage comprising a polypeptide, and wherein said Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which can be directly detected when attached to said phosphate via said polypeptide chemical linkage or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide.

1192. (Canceled)

1193. (Currently Amended) The oligo- or polynucleotide of claim 1191, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

1194. (Previously Added) The oligo- or polynucleotide of claim 1193, wherein said magnetic component comprises magnetic oxide.

1195. (Previously Added) The oligo- or polynucleotide of claim 1194, wherein said magnetic oxide comprises ferric oxide.

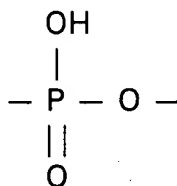
1196. (Previously Added) The oligo- or polynucleotide of claim 1193, wherein said metal-containing component is catalytic.

1197. (Previously Added) The oligo- or polynucleotide of claim 1193, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

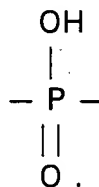
1198. (Canceled)

1199. (Canceled)

1200. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said covalent attachment comprises



or

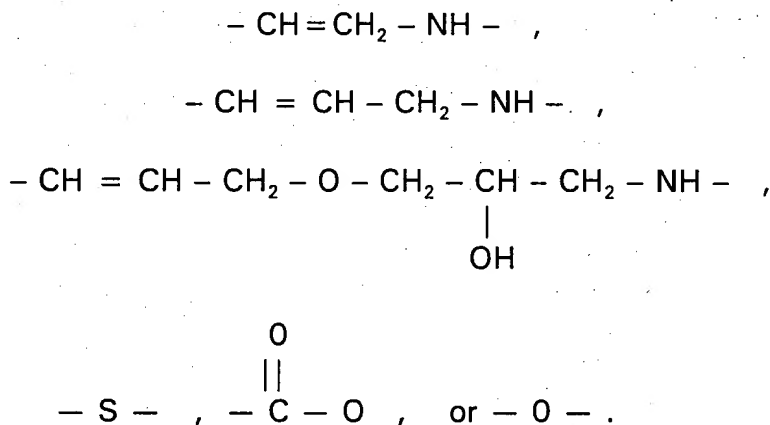


1201. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said polypeptide chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

1202. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said polypeptide chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

1203. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said polypeptide chemical linkage comprises an allylamine group.

1204. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said polypeptide chemical linkage comprises any of the moieties:



1205. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said polypeptide chemical linkage comprises a glycosidic linkage moiety.

1206. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said x comprises a monophosphate, a diphosphate or a triphosphate and y comprises a monophosphate.

1207. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said Sig moiety is covalently attached to said phosphate through a phosphorus atom or phosphate oxygen.

1208. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said x comprises a monophosphate.

1209. (Currently Amended) The oligo- or polynucleotide of claim 1191, wherein said Sig moiety is attached via said polypeptide chemical linkage to the furanosyl phosphate moiety of a terminal nucleotide in said oligo- or polynucleotide.

1210. (Previously Added) The oligo- or polynucleotide of claim 1209, wherein z of said furanosyl moiety of said terminal nucleotide comprises a hydrogen atom.

1211. (Previously Added) The oligo- or polynucleotide of claim 1209, wherein z of said furanosyl moiety of said terminal nucleotide comprises an oxygen atom.

1212. (Previously Added) The oligo- or polynucleotide of claim 1210, wherein y of said furanosyl moiety comprises a hydrogen atom.

1213. (Previously Added) The oligo- or polynucleotide of claim 1211, wherein y of said furanosyl moiety comprises an oxygen atom.

1214. (Canceled)

1215. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

1216. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

1217. (Previously Added) The oligo- or polynucleotide of claim 1216, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

1218. (Previously Added) The oligo- or polynucleotide of claim 1216, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

1219. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs, or a combination of any of the foregoing.

1220. (Previously Added) The oligo- or polynucleotide of claim 1219, wherein said adenosine analogs comprise tubercidin and toyocamycin.

1221. (Previously Added) The oligo- or polynucleotide of claim 1191, further comprising at least one ribonucleotide.

1222. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

1223. (Currently Amended) The oligo- or polynucleotide of claim ~~4222~~ 1191, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

1224. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

1225. (Currently Amended) The oligo- or polynucleotide of claim ~~1224~~ 1191, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one ~~deoxyribonucleotide~~ deoxyribonucleotide.

1226. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said polypeptide chemical linkage comprises polylysine.

1227. (Previously Added) The oligo- or polynucleotide of claim 1191, wherein said polypeptide chemical linkage comprises avidin, streptavidin or anti-hapten immunoglobulin.

* * * * *

828. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 826, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

829. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 828, wherein said magnetic component comprises magnetic oxide.

830. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 829, wherein said magnetic oxide comprises ferric oxide.

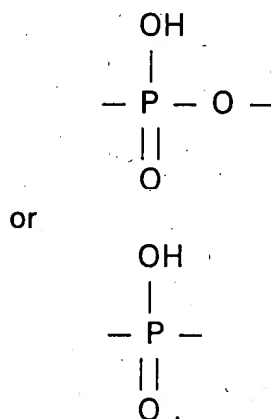
831. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 828, wherein said metal-containing component is catalytic.

832. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 828, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

833. (Canceled)

834. (Canceled)

835. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said covalent attachment comprises

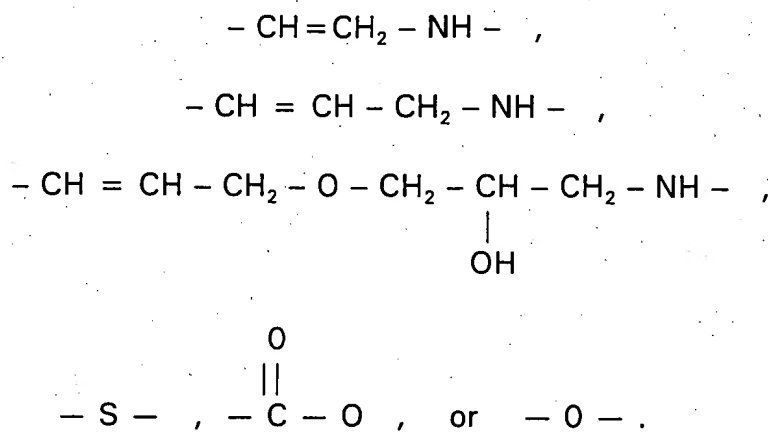


836. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

837. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

838. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said chemical linkage comprises an allylamine group.

839. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said chemical linkage comprises any of the moieties:



840. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said chemical linkage comprises a glycosidic linkage moiety.

841. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said PM comprises a monophosphate, a diphosphate or a triphosphate.

842. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oxygen.

843. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 826, wherein said Sig moiety is attached to the ~~furanosyl moiety~~ PM of a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

844. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 843, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

845. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 843, wherein the furanosyl moiety of said terminal nucleotide ~~has~~ comprises an oxygen atom at the 2' position thereof.

846. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 844, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 3' position thereof.

847. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 845, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 3' position thereof.

848. (Canceled)

849. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

850. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

851. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 850, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

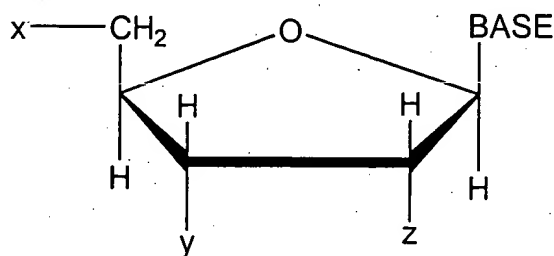
852. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 850, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

853. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 826, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs or a combination of any of the foregoing.

854. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 853, wherein said adenosine analogs comprise tubericidin or toyocamycin.

855. (Currently Amended) An oligo- or polynucleotide comprising the ~~The~~ oligo- or polydeoxyribonucleotide of claim 826, and further comprising at least one ribonucleotide.

856. (Currently Amended) An oligo- or polydeoxyribonucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide or modified nucleotide analog having the structural formula:



wherein BASE is a moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, and wherein said BASE is attached to the 1' position of the furanosyl ring from the N1 position when said BASE is a pyrimidine or a pyrimidine analog, or from the N9 position of the furanosyl ring when said BASE is a purine, a purine analog, a deazapurine or a deazapurine analog;

wherein x comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein y comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein z comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate; and

wherein Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which comprises at least three carbon atoms and is covalently attached directly or through a chemical linkage to at least one phosphate comprising x, y, z, or a combination thereof, and wherein said Sig ~~comprises a non-polypeptide, non-~~

~~nucleotidyl, non-radioactive-label moiety~~ which can be directly or indirectly detected when attached to said phosphate or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polydeoxyribonucleotide or when said oligo- or polydeoxyribonucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof.

857. (Canceled)

858. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 856, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

859. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 858, wherein said magnetic component comprises magnetic oxide.

860. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 859, wherein said magnetic oxide comprises ferric oxide.

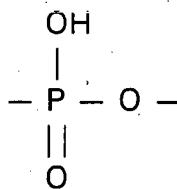
861. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 858, wherein said metal-containing component is catalytic.

862. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 858, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

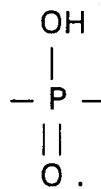
863. (Canceled)

864. (Canceled)

865. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856,
wherein said covalent attachment comprises



or

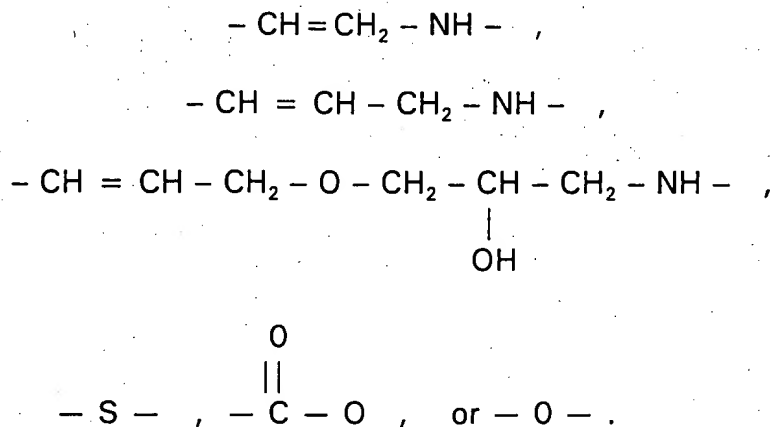


866. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856,
wherein said chemical linkage does not interfere substantially with the
characteristic ability of Sig to form a detectable signal.

867. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856,
wherein said chemical linkage comprises a -CH₂NH- moiety.

868. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856,
wherein said chemical linkage comprises an allylamine group.

869. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said chemical linkage comprises any of the moieties:



870. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said chemical linkage comprises a glycosidic linkage moiety.

871. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said x comprises a monophosphate, a diphosphate or a triphosphate and y comprises a monophosphate.

872. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said Sig moiety is covalently attached to said phosphate through a phosphorus atom or phosphate oxygen.

873. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said x comprises a monophosphate.

874. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 856, wherein said Sig moiety is attached to the ~~furanosyl~~ phosphate moiety of a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

875. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 874, wherein z of said furanosyl moiety of said terminal nucleotide comprises a hydrogen atom.

876. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 874, wherein z of said furanosyl moiety of said terminal nucleotide comprises an oxygen atom.

877. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 875, wherein y of said furanosyl moiety comprises a hydrogen atom.

878. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 876, wherein y of said furanosyl moiety comprises an oxygen atom.

879. (Canceled)

880. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

881. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

882. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 881, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

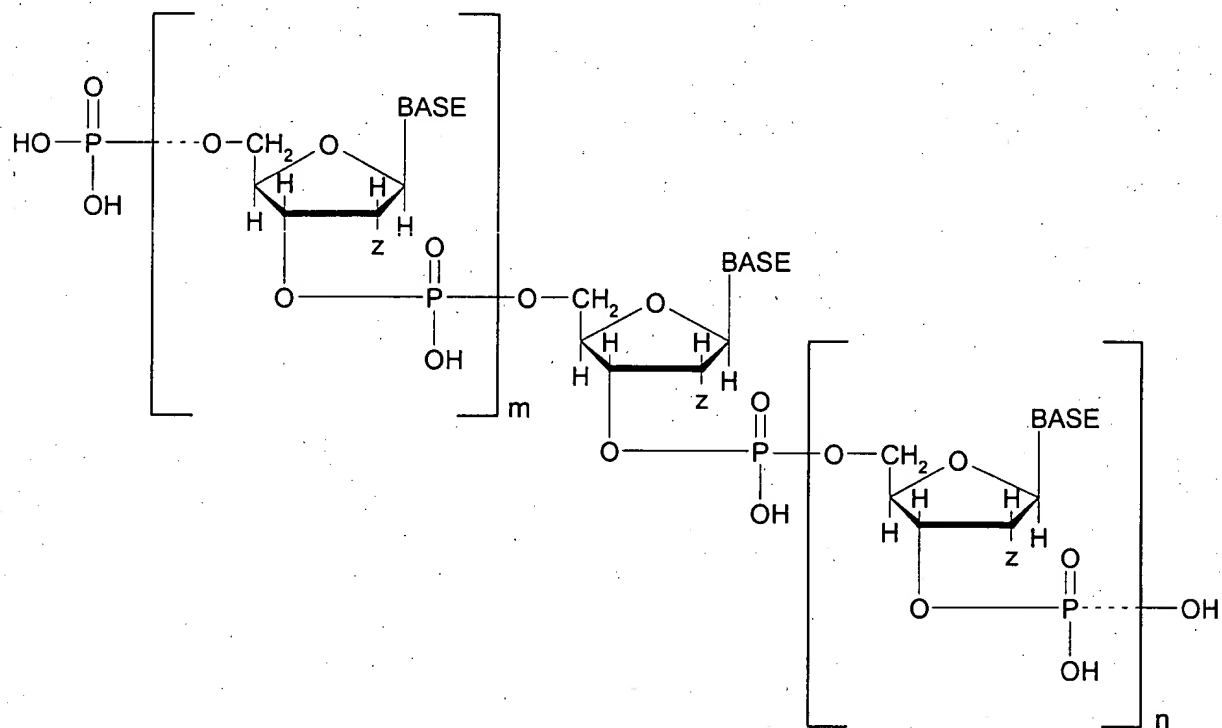
883. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 881, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

884. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 856, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs, or a combination of any of the foregoing.

885. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 884, wherein said adenosine analogs comprise tubercidin or toyocamycin.

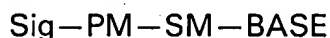
886. (Currently Amended) An oligo- or polynucleotide comprising the ~~The~~ oligo- or polydeoxyribonucleotide of claim 856, and further comprising at least one ribonucleotide.

887. (Currently Amended) The oligo- or ~~polydeoxyribonucleotide~~
polydeoxyribonucleotide of claim 856, having the structural formula:



wherein m and n represent integers from 0 up to about 100,000, and wherein said
 Sig moiety is attached to at least one of the phosphate moieties in said structural
 formula.

888. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide modified nucleotide analog having the formula



wherein PM is a phosphate moiety, SM is a furanosyl moiety and BASE is a base moiety or a base analog comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, wherein said PM is attached to SM, said BASE is attached to SM, said Sig is covalently attached to PM directly or through a chemical linkage, ~~non-nucleotidyl~~, and wherein said Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety comprising at least three carbon atoms and which Sig can be directly or indirectly detected when attached to PM or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide, and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide.

889. (Canceled)

890. (Currently Amended) The oligo- or polynucleotide of claim 888, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

891. (Previously Added) The oligo- or polynucleotide of claim 890, wherein said magnetic component comprises magnetic oxide.

892. (Previously Added) The oligo- or polynucleotide of claim 891, wherein said magnetic oxide comprises ferric oxide.

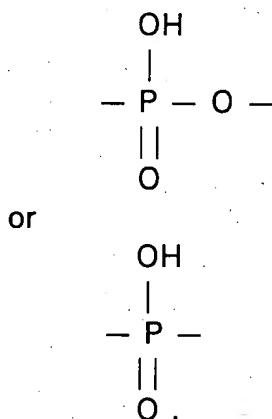
893. (Previously Added) The oligo- or polynucleotide of claim 890, wherein said metal-containing component is catalytic.

894. (Previously Added) The oligo- or polynucleotide of claim 890, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

895. (Canceled)

896. (Canceled)

897. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said covalent attachment comprises:

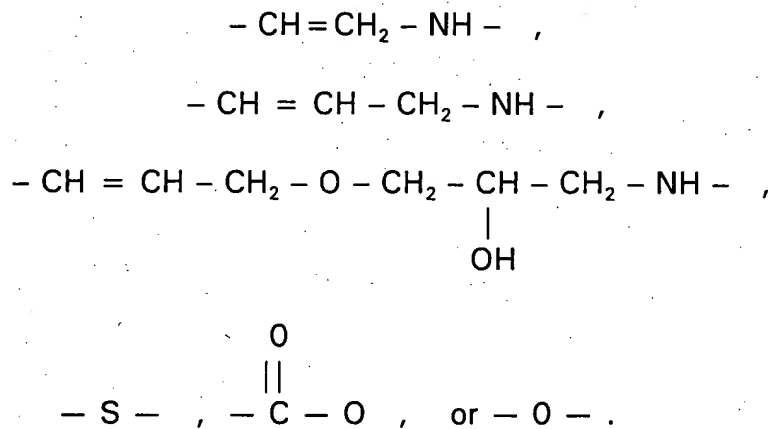


898. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

899. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said chemical linkage comprises a -CH₂NH- moiety.

900. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said chemical linkage comprises an allylamine group.

901. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said chemical linkage comprises any of the moieties:



902. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said chemical linkage comprises a glycosidic linkage moiety.

903. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said PM comprises a monophosphate, a diphosphate or a triphosphate.

904. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oxygen.

905. (Currently Amended) The oligo- or polynucleotide of claim 888, wherein said Sig moiety is attached to the furanosyl moiety PM of a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

906. (Previously Added) The oligo- or polynucleotide of claim 905, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

907. (Previously Added) The oligo- or polynucleotide of claim 905, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 2' position thereof.

908. (Previously Added) The oligo- or polynucleotide of claim 906, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 3' position thereof.

909. (Previously Added) The oligo- or polynucleotide of claim 907, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 3' position thereof.

910. (Canceled)

911. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

912. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

913. (Previously Added) The oligo- or polynucleotide of claim 912, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

914. (Previously Added) The oligo- or polynucleotide of claim 912, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

915. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs or a combination of any of the foregoing.

916. (Previously Added) The oligo- or polynucleotide of claim 915, wherein said adenosine analogs comprise tubericidin or toyocamycin.

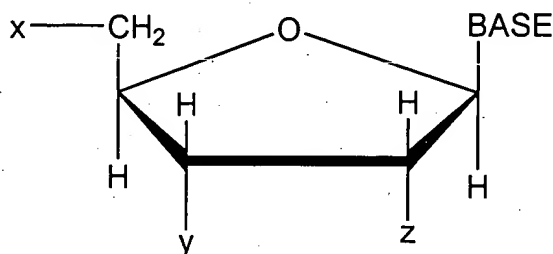
917. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

918. (Currently Amended) The oligo- or polynucleotide of claim ~~917~~ 888, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

919. (Previously Added) The oligo- or polynucleotide of claim 888, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

920. (Currently Amended) The oligo- or polynucleotide of claim ~~919~~ 888, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one ~~deoxyribonucleotide~~ deoxyribonucleotide.

921. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide or modified nucleotide analog having the structural formula:



wherein BASE is a moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said pyrimidine analog, said purine analog or said deazapurine analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, wherein said BASE is attached to the 1' position of the furanosyl ring from the N1 position when said BASE is a pyrimidine or pyrimidine analog, or from the N9 position of the furanosyl ring when said BASE is a purine, purine analog, a deazapurine or a deazapurine analog;

wherein x comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein y comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein z comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate; and

wherein Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which comprises at least three carbon atoms, wherein Sig is covalently attached directly or through a chemical linkage to at least one phosphate comprising x, y and z, or a combination thereof, and wherein said Sig comprises a

~~non polypeptide, non nucleotidyl, non radioactive label moiety~~ which can be directly or indirectly detected when so attached to said phosphate or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide.

922. (Canceled)

923. (Currently Amended) The oligo- or polynucleotide of claim 921, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

924. (Previously Added) The oligo- or polynucleotide of claim 923, wherein said magnetic component comprises magnetic oxide.

925. (Previously Added) The oligo- or polynucleotide of claim 924, wherein said magnetic oxide comprises ferric oxide.

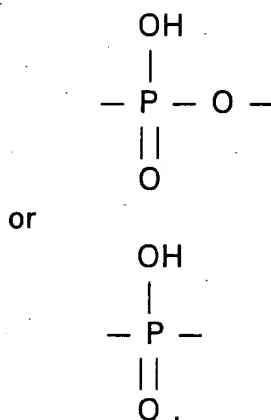
926. (Previously Added) The oligo- or polynucleotide of claim 923, wherein said metal-containing component is catalytic.

927. (Previously Added) The oligo- or polynucleotide of claim 923, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

928. (Canceled)

929. (Canceled)

930. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said covalent attachment comprises

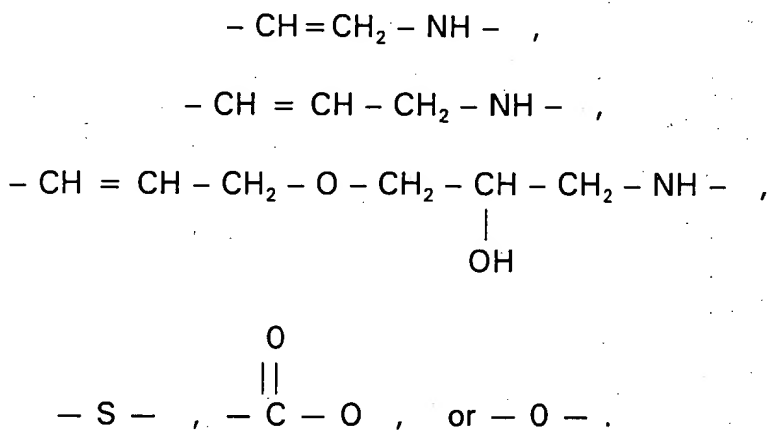


931. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

932. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said chemical linkage comprises a -CH₂NH- moiety.

933. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said chemical linkage comprises an allylamine group.

934. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said chemical linkage comprises any of the moieties:



935. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said chemical linkage comprises a glycosidic linkage moiety.

936. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said x comprises a monophosphate, a diphosphate or a triphosphate and y comprises a monophosphate.

937. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said Sig moiety is covalently attached to said phosphate through a phosphorus atom or phosphate oxygen.

938. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said x comprises a monophosphate.

939. (Currently Amended) The oligo- or polynucleotide of claim 921, wherein said Sig moiety is attached to the ~~furanosyl~~ phosphate moiety of a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

940. (Previously Added) The oligo- or polynucleotide of claim 939, wherein z of said furanosyl moiety of said terminal nucleotide comprises a hydrogen atom.

941. (Previously Added) The oligo- or polynucleotide of claim 939, wherein z of said furanosyl moiety of said terminal nucleotide comprises an oxygen atom.

942. (Previously Added) The oligo- or polynucleotide of claim 940, wherein y of said furanosyl moiety comprises a hydrogen atom.

943. (Previously Added) The oligo- or polynucleotide of claim 941, wherein y of said furanosyl moiety comprises an oxygen atom.

944. (Canceled)

945. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

946. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

947. (Previously Added) The oligo- or polynucleotide of claim 946, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

948. (Previously Added) The oligo- or polynucleotide of claim 946, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

949. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs, or a combination of any of the foregoing.

950. (Previously Added) The oligo- or polynucleotide of claim 949, wherein said adenosine analogs comprise tubercidin or toyocamycin.

951. (Previously Added) The oligo- or polynucleotide of claim 921, further comprising at least one ribonucleotide.

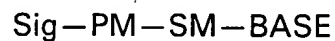
952. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

953. (Currently Amended) The oligo- or polynucleotide of claim ~~952~~ 921, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

954. (Previously Added) The oligo- or polynucleotide of claim 921, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

955. (Currently Amended) The oligo- or polynucleotide of claim ~~954~~ 921, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one deoxyribonucleotide.

956. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide or modified nucleotide analog having the formula



wherein PM is a phosphate moiety, SM is a ~~sugar~~ furanosyl moiety and BASE is a base moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog wherein said analog can be attached to or coupled to or incorporated into DNA or RNA wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, said PM being attached to SM, said BASE being attached to SM, and said Sig being covalently attached to PM directly or through a chemical linkage, ~~and~~ wherein said Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety comprising at least three carbon atoms and which Sig can be directly or indirectly detected when attached to PM or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, and wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, a saccharide component or a combination of any of the foregoing.

957. (Canceled)

958. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said magnetic component comprises magnetic oxide.

959. (Previously Added) The oligo- or polynucleotide of claim 958, wherein said magnetic oxide comprises ferric oxide.

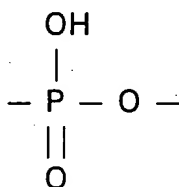
960. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said metal-containing component is catalytic.

961. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

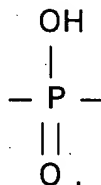
962. (Canceled)

963. (Canceled)

964. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said covalent attachment comprises



or

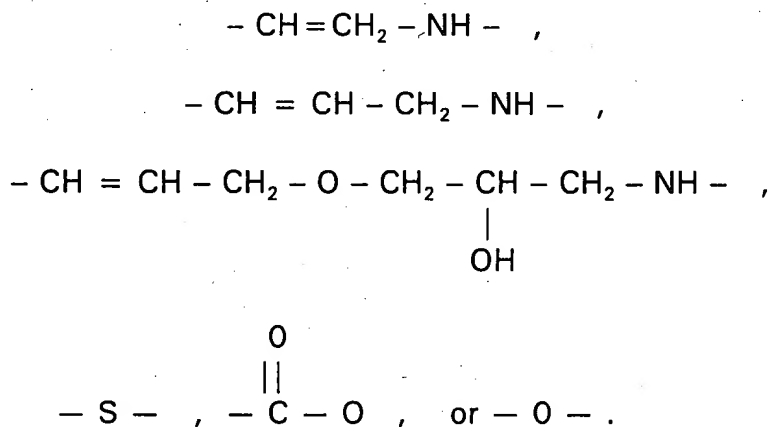


965. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

966. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

967. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said chemical linkage comprises an allylamine group.

968. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said chemical linkage comprises any of the moieties:



969. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said chemical linkage comprises a glycosidic linkage moiety.

970. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said PM comprises a monophosphate, a diphosphate or a triphosphate.

971. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oxygen.

972. (Currently Amended) The oligo- or polynucleotide of claim 956, wherein said Sig moiety is attached to the ~~furanosyl moiety~~ PM of a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

973. (Previously Added) The oligo- or polynucleotide of claim 972, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

974. (Previously Added) The oligo- or polynucleotide of claim 972, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 2' position thereof.

975. (Previously Added) The oligo- or polynucleotide of claim 973, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 3' position thereof.

976. (Previously Added) The oligo- or polynucleotide of claim 974, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 3' position thereof.

977. (Canceled)

978. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

979. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

980. (Previously Added) The oligo- or polynucleotide of claim 979, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

981. (Previously Added) The oligo- or polynucleotide of claim 979, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

982. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs or a combination of any of the foregoing.

983. (Previously Added) The oligo- or polynucleotide of claim 982, wherein said adenosine analogs comprise tubericidin and toyocamycin.

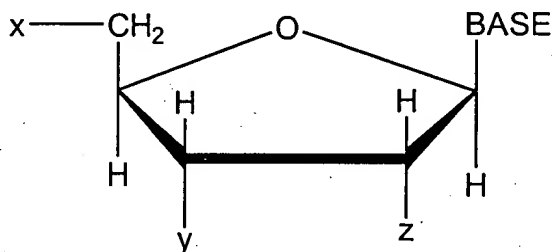
984. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

985. (Currently Amended) The oligo- or polynucleotide of claim ~~984~~ 956, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

986. (Previously Added) The oligo- or polynucleotide of claim 956, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

987. (Currently Amended) The oligo- or polynucleotide of claim ~~986~~ 956, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one ~~deoxyribonucleotide~~ deoxyribonucleotide.

988. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide or a modified nucleotide analog having the structural formula:



wherein BASE is a moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog, can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, and wherein said BASE is attached to the 1' position of the furanosyl ring from the N1 position when said BASE is a pyrimidine or a pyrimidine analog, or from the N9 position when said BASE is a purine, a purine analog, a deazapurine or a deazapurine analog;

wherein x comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein y comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein z comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate; and

wherein Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which comprises at least three carbon atoms and which Sig is covalently attached directly or through a chemical linkage to at least one phosphate comprising x, y, z, or a combination thereof, and wherein said Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which can be directly or indirectly detected when attached to said phosphate or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, a saccharide component or a combination of any of the foregoing.

989. (Canceled)

990. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said magnetic component comprises magnetic oxide.

991. (Previously Added) The oligo- or polynucleotide of claim 990, wherein said magnetic oxide comprises ferric oxide.

992. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said

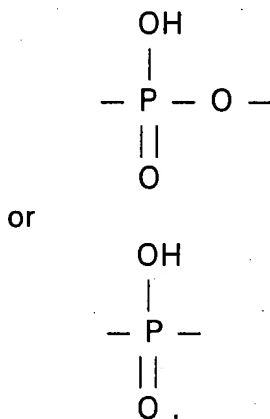
metal-containing component is catalytic.

993. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

994. (Canceled)

995. (Canceled)

996. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said covalent attachment comprises

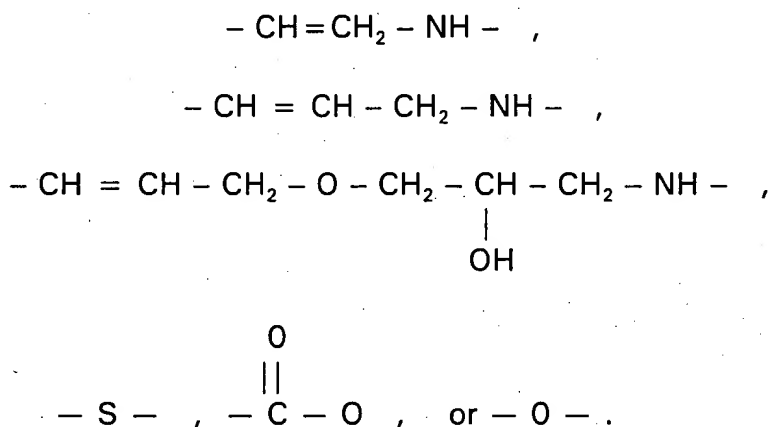


997. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

998. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said chemical linkage comprises a -CH₂NH- moiety.

999. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said chemical linkage comprises an allylamine group.

1000. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said chemical linkage comprises any of the moieties:



1001. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said chemical linkage comprises a glycosidic linkage moiety.

1002. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said x comprises a monophosphate, a diphosphate or a triphosphate and y comprises a monophosphate.

1003. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said Sig moiety is covalently attached to said phosphate through a phosphorus atom or phosphate oxygen.

1004. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said x comprises a monophosphate.

1005. (Currently Amended) The oligo- or polynucleotide of claim 988, wherein said Sig moiety is attached to the ~~furanosyl~~ phosphate moiety of a terminal nucleotide in said oligo- or polynucleotide.

1006. (Previously Added) The oligo- or polynucleotide of claim 1005, wherein z of said furanosyl moiety of said terminal nucleotide comprises a hydrogen atom.

1007. (Previously Added) The oligo- or polynucleotide of claim 1005, wherein z of said furanosyl moiety of said terminal nucleotide comprises an oxygen atom.

1008. (Previously Added) The oligo- or polynucleotide of claim 1006, wherein y of said furanosyl moiety comprises a hydrogen atom.

1009. (Previously Added) The oligo- or polynucleotide of claim 1007, wherein y of said furanosyl moiety comprises an oxygen atom.

1010. (Canceled)

1011. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

1012. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

1013. (Previously Added) The oligo- or polynucleotide of claim 1012, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

1014. (Previously Added) The oligo- or polynucleotide of claim 1012, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

1015. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs, or a combination of any of the foregoing.

1016. (Previously Added) The oligo- or polynucleotide of claim 1015, wherein said adenosine analogs comprise tubericidin or toyocamycin.

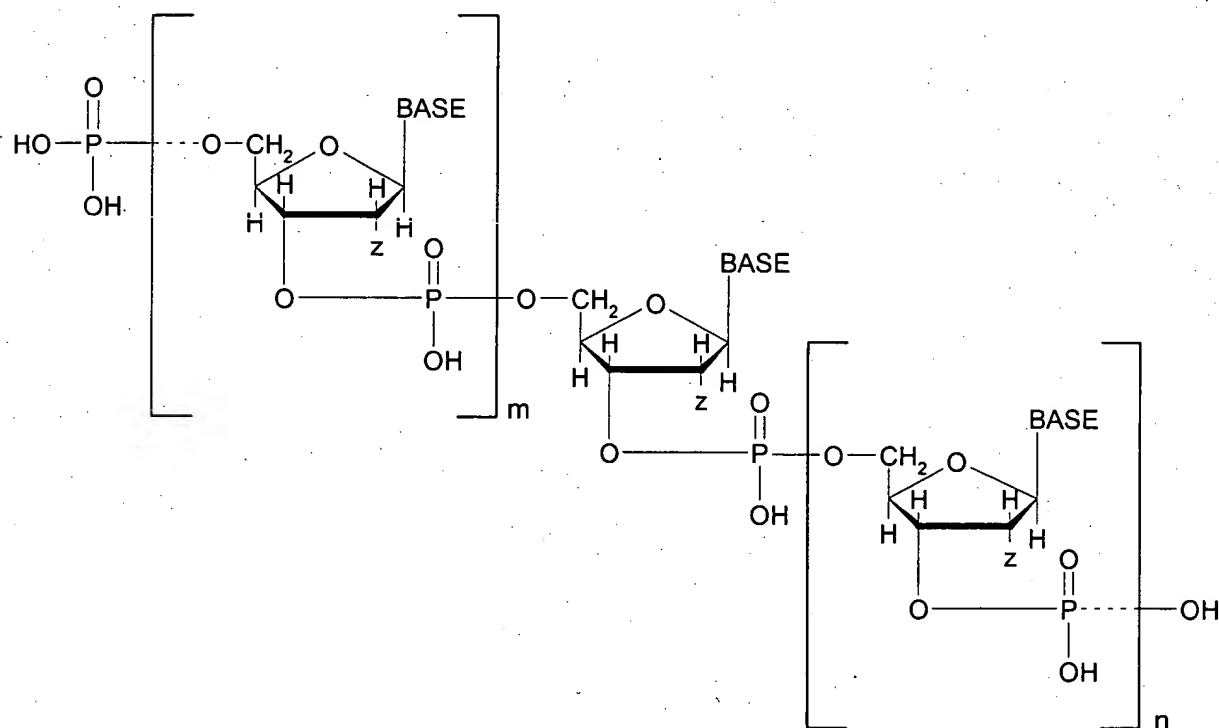
1017. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

1018. (Currently Amended) The oligo- or polynucleotide of claim ~~4017~~ 988, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

1019. (Previously Added) The oligo- or polynucleotide of claim 988, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

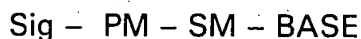
1020. (Currently Amended) The oligo- or polynucleotide of claim ~~4019~~ 988, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one ~~deoxyribonucleotide~~ deoxyribonucleotide.

1021. (Previously Added) The oligo- or polynucleotide of claim 988, having the structural formula:



wherein m and n represent integers from 0 up to about 100,000, and wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

1022. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide or a modified nucleotide analog having the formula



wherein PM is a phosphate moiety, SM is a furanosyl moiety and BASE is a base moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, said PM is attached to SM, said BASE is attached to SM, said Sig is covalently attached to PM directly or via a chemical linkage, and wherein said Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety comprising at least three carbon atoms and which Sig which can be directly or indirectly detected when attached to PM or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide, and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide, and wherein said Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

1023. (Canceled)

1024. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said magnetic component comprises magnetic oxide.

1025. (Previously Added) The oligo- or polynucleotide of claim 1024, wherein said magnetic oxide comprises ferric oxide.

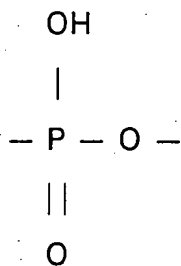
1026. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said metal-containing component is catalytic.

1027. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

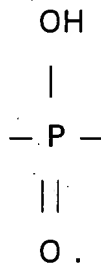
1028. (Canceled)

1029. (Canceled)

1030. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said covalent attachment comprises



or

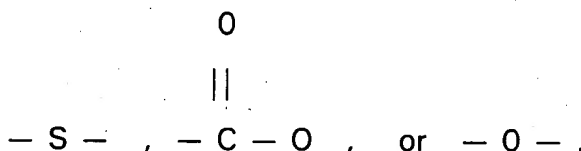
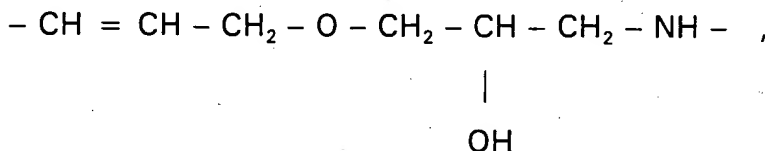
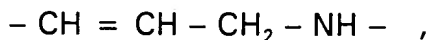
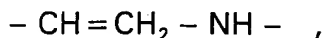


1031. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

1032. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

1033. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said chemical linkage comprises an allylamine group.

1034. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said chemical linkage comprises any of the moieties:



1035. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said chemical linkage comprises a glycosidic linkage moiety.

1036. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said PM comprises a monophosphate, a diphosphate or a triphosphate.

1037. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oxygen.

1038. (Currently Amended) The oligo- or polynucleotide of claim 1022, wherein said Sig moiety is attached to the furanosyl moiety PM of a terminal nucleotide in said oligo- or polynucleotide.

1039. (Previously Added) The oligo- or polynucleotide of claim 1038, wherein the furanosyl moiety of said terminal nucleotide has a hydrogen atom at the 2' position thereof.

1040. (Previously Added) The oligo- or polynucleotide of claim 1038, wherein the furanosyl moiety of said terminal nucleotide has an oxygen atom at the 2' position thereof.

1041. (Previously Added) The oligo- or polynucleotide of claim 1039, wherein the furanosyl moiety of said terminal nucleotide has a hydrogen atom at the 3' position thereof.

1042. (Previously Added) The oligo- or polynucleotide of claim 1040, wherein the furanosyl moiety of said terminal nucleotide has an oxygen atom at the 3' position thereof.

1043. (Canceled)

1044. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

1045. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

1046. (Previously Added) The oligo- or polynucleotide of claim 1045, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

1047. (Previously Added) The oligo- or polynucleotide of claim 1045, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

1048. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs or a combination of any of the foregoing.

1049. (Previously Added) The oligo- or polynucleotide of claim 1048, wherein said adenosine analogs comprise tubericidin and toyocamycin.

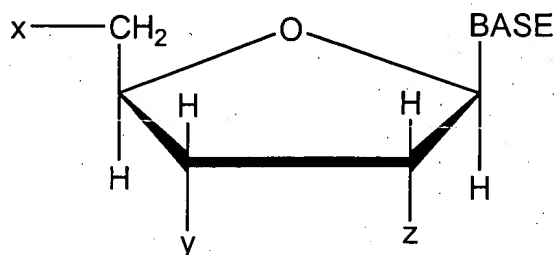
1050. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

1051. (Currently Amended) The oligo- or polynucleotide of claim ~~1050~~ 1022, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

1052. (Previously Added) The oligo- or polynucleotide of claim 1022, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

1053. (Currently Amended) The oligo- or polynucleotide of claim ~~1052~~ 1022, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one ~~deoxyribonucleotide~~ deoxyribonucleotide.

1054. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide or a modified nucleotide analog having the structural formula:



wherein BASE is a moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine, a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, and wherein BASE is attached to the 1' position of the furanosyl ring from the N1 position when BASE is a pyrimidine or a pyrimidine analog, from the N9 position of the furanosyl ring when BASE is a purine, a purine analog, a deazapurine or a deazapurine;

wherein x comprises of H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein y comprises of H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein z comprises of H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate; and

wherein Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which comprises at least three carbon atoms, wherein said Sig is covalently attached directly or through a chemical linkage to at least one phosphate comprising of x, y and z, or a combination thereof, and wherein said Sig ~~comprises~~ a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which can be directly or indirectly detected when so attached to said phosphate or when said

modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide, and wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~, or a combination of any of the foregoing.

1055. (Canceled)

1056. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said magnetic component comprises magnetic oxide.

1057. (Previously Added) The oligo- or polynucleotide of claim 1056, wherein said magnetic oxide comprises ferric oxide.

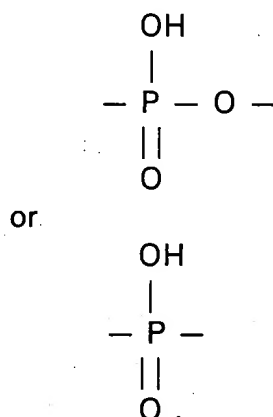
1058. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said metal-containing component is catalytic.

1059. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

1060. (Canceled)

1061. (Canceled)

1062. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said covalent attachment comprises

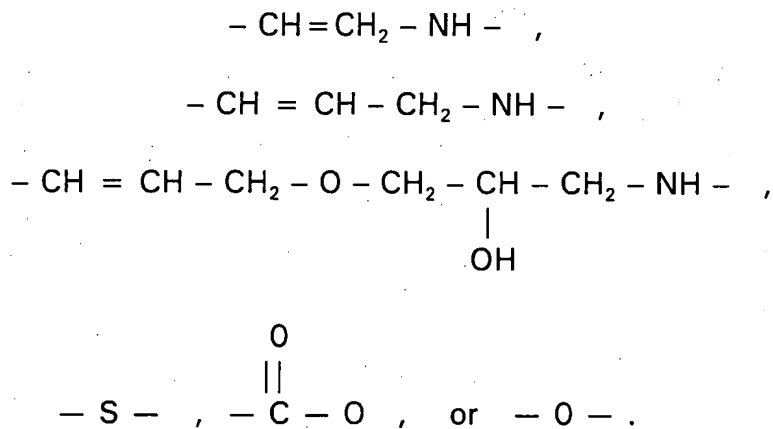


1063. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

1064. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

1065. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said chemical linkage comprises an allylamine group.

1066. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said chemical linkage comprises any of the moieties:



1067. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said chemical linkage comprises a glycosidic linkage moiety.

1068. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said x comprises a monophosphate, a diphosphate or a triphosphate and y comprises a monophosphate.

1069. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said Sig moiety is covalently attached to said phosphate through a phosphorus atom or phosphate oxygen.

1070. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said x comprises a monophosphate.

1071. (Currently Amended) The oligo- or polynucleotide of claim 1054, wherein said Sig moiety is attached to the ~~furanosyl~~ phosphate moiety of a terminal nucleotide in said oligo- or polynucleotide.

1072. (Previously Added) The oligo- or polynucleotide of claim 1071, wherein z of said furanosyl moiety of said terminal nucleotide comprises a hydrogen atom.

1073. (Previously Added) The oligo- or polynucleotide of claim 1071, wherein z of said furanosyl moiety of said terminal nucleotide comprises an oxygen atom.

1074. (Previously Added) The oligo- or polynucleotide of claim 1072, wherein y of said furanosyl moiety comprises a hydrogen atom.

1075. (Previously Added) The oligo- or polynucleotide of claim 1073, wherein y of said furanosyl moiety comprises an oxygen atom.

1076. (Canceled)

1077. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

1078. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

1079. (Previously Added) The oligo- or polynucleotide of claim 1078, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

1080. (Previously Added) The oligo- or polynucleotide of claim 1078, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

1081. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs, or a combination of any of the foregoing.

1082. (Previously Added) The oligo- or polynucleotide of claim 1081, wherein said adenosine analogs comprise tubercidin or toyocamycin.

1083. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide.

1084. (Currently Amended) The oligo- or polynucleotide of claim ~~1083~~ 1054, wherein said oligo- or polynucleotide comprises an oligo- or polydeoxyribonucleotide and further comprises at least one ribonucleotide.

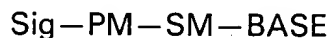
1085. (Previously Added) The oligo- or polynucleotide of claim 1054, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide.

1086. (Currently Amended) The oligo- or polynucleotide of claim ~~1085~~ 1054, wherein said oligo- or polynucleotide comprises an oligo- or polyribonucleotide and further comprises at least one ~~deoxyribonucleotide~~ deoxyribonucleotide.

The diagram illustrates a branched nucleic acid polymer. The main chain is represented by a series of nucleotides enclosed in large brackets with a subscript m . Each nucleotide in the main chain consists of a sugar (ribose or deoxyribose) and a nitrogenous base (labeled "BASE"). The sugar is connected to the phosphate group of the next nucleotide via an oxygen atom at the 3' position. The phosphate group is connected to the sugar of the next nucleotide via an oxygen atom at the 5' position. A side chain branches off from the main chain at the 3' position of one of the nucleotides. This side chain is also enclosed in brackets with a subscript n and consists of a sugar and a phosphate group. The side chain sugar is connected to the main chain sugar via an oxygen atom at the 3' position. The side chain phosphate group is connected to the side chain sugar via an oxygen atom at the 5' position. The main chain phosphate group is connected to the main chain sugar via an oxygen atom at the 5' position. The side chain phosphate group is connected to the side chain sugar via an oxygen atom at the 5' position. The side chain phosphate group is also connected to the main chain phosphate group via an oxygen atom at the 3' position.

Enz-5(D6)(C2)

1088. (Currently Amended) An oligo- or polydeoxyribonucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide or a modified nucleotide analog having the formula



wherein PM is a phosphate moiety, SM is a furanosyl moiety and BASE is a base moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, wherein said PM is attached to SM, said BASE is attached to SM, said Sig is covalently attached to PM through a chemical linkage comprising a polypeptide, and wherein said Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety comprising at least three carbon atoms and which Sig can be directly detected when indirectly attached to PM through said polypeptide chemical linkage or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polydeoxyribonucleotide or when said oligo- or polydeoxyribonucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof.

1089. (Canceled)

1090. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 1088, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

1091. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1090, wherein said magnetic component comprises magnetic oxide.

1092. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1091, wherein said magnetic oxide comprises ferric oxide.

1093. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1090, wherein said metal-containing component is catalytic.

1094. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1090, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

1095. (Canceled)

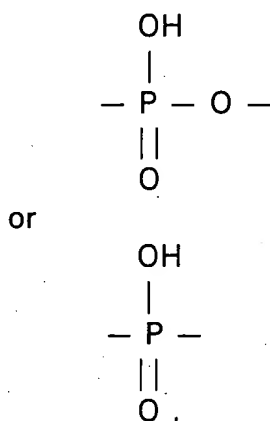
1096. (Canceled)

1097. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said polypeptide chemical linkage comprises polylysine.

1098. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said polypeptide chemical linkage comprises avidin, streptavidin or anti-hapten immunoglobulin.

1099. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said Sig moiety is attached via said polypeptide chemical linkage to a phosphate moiety in a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

1100. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said covalent attachment comprises

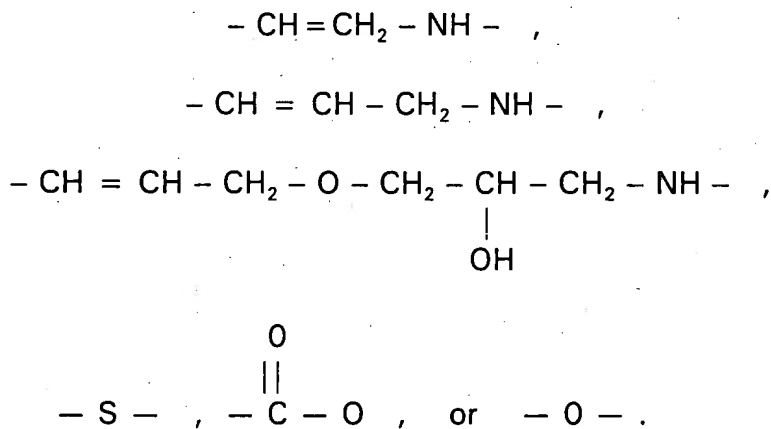


1101. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said polypeptide chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

1102. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said polypeptide chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

1103. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said polypeptide chemical linkage comprises an allylamine group.

1104. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said polypeptide chemical linkage further comprises any of the moieties:



1105. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said polypeptide chemical linkage comprises a glycosidic linkage moiety.

1106. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said PM comprises a monophosphate, a diphosphate or a triphosphate.

1107. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said Sig moiety is covalently indirectly attached to said PM through a phosphorus atom or phosphate oxygen.

1108. (Currently Amended)- The oligo- or polydeoxyribonucleotide of claim 1088, wherein said Sig moiety is attached via said polypeptide chemical linkage to a ~~furanosyl moiety~~ PM in a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

1109. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1108, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

1110. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1108, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 2' position thereof.

1111. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1109, wherein the furanosyl moiety of said terminal nucleotide comprises a hydrogen atom at the 3' position thereof.

1112. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1110, wherein the furanosyl moiety of said terminal nucleotide comprises an oxygen atom at the 3' position thereof.

1113. (Canceled)

1114. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

1115. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

1116. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1115, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

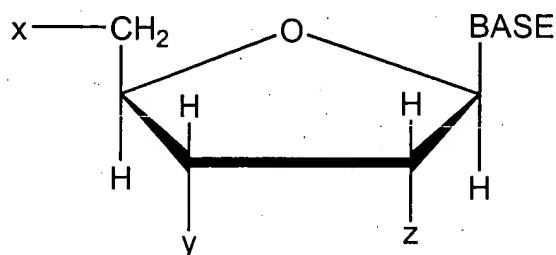
1117. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1115, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

1118. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1088, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs or a combination of any of the foregoing.

1119. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1118, wherein said adenosine analogs comprise tubericidin and toyocamycin.

1120. (Currently Amended) An oligo- or polynucleotide comprising the ~~The~~ oligo- or polydeoxyribonucleotide of claim 1088, and further comprising at least one ribonucleotide.

1121. (Currently Amended) An oligo- or polydeoxyribonucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide or modified analog having the structural formula:



wherein BASE is a moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, and wherein said BASE is attached to the 1' position of the furanosyl ring from the N1 position when said BASE is a pyrimidine or a pyrimidine analog, or from the N9 position of the furanosyl ring when said BASE is a purine, a purine analog, a deazapurine or a deazapurine analog;

wherein x comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein y comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate;

wherein z comprises H—, HO—, a mono-phosphate, a di-phosphate or a tri-phosphate; and

wherein Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety which comprises at least three carbon atoms and is covalently attached through a chemical linkage to at least one phosphate comprising x, y, z, or a combination thereof, wherein said chemical linkage comprises a polypeptide, and wherein said Sig ~~comprises a non-polypeptide, non-nucleotidyl non-radioactive label moiety which~~ can be directly or indirectly detected when attached to said

phosphate via said polypeptide chemical linkage, or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polydeoxynucleotide, or when said oligo- or polydeoxynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof.

1122. (Canceled)

1123. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 1121, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

1124. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1123, wherein said magnetic component comprises magnetic oxide.

1125. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1124, wherein said magnetic oxide comprises ferric oxide.

1126. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1123, wherein said metal-containing component is catalytic.

1127. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1123, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

1128. (Canceled)

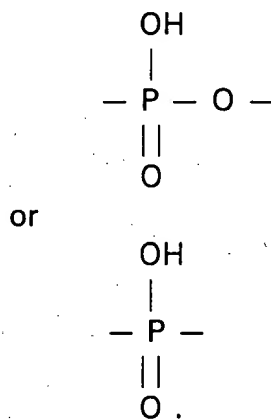
1129. (Canceled)

1130. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said polypeptide chemical linkage comprises polylysine.

1131. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said polypeptide chemical linkage comprises avidin, streptavidin or anti-hapten immunoglobulin.

1132. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said Sig moiety is attached via said polypeptide chemical linkage to a phosphate moiety in a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

1133. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said covalent attachment comprises

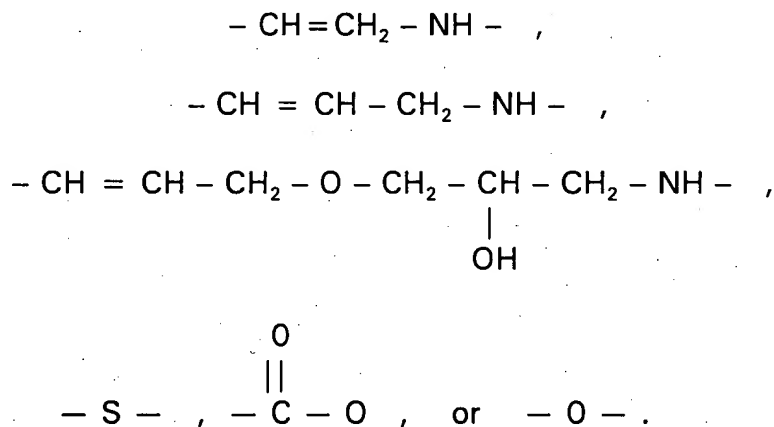


1134. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said polypeptide chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

1135. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said polypeptide chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

1136. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said polypeptide chemical linkage comprises an allylamine group.

1137. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said polypeptide chemical linkage further comprises any of the moieties:



1138. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said polypeptide chemical linkage comprises a glycosidic linkage moiety.

1139. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said x comprises a monophosphate, a diphosphate or a triphosphate and y comprises a monophosphate.

1140. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said Sig moiety is covalently indirectly attached to said phosphate through a phosphorus atom or phosphate oxygen.

1141. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said x comprises a monophosphate.

1142. (Currently Amended) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said Sig moiety is attached via said polypeptide chemical linkage to the furanosyl phosphate moiety of a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

1143. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1142, wherein z of said furanosyl moiety of said terminal nucleotide comprises a hydrogen atom.

1144. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1142, wherein z of said furanosyl moiety of said terminal nucleotide comprises an oxygen atom.

1145. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1143, wherein y of said furanosyl moiety comprises a hydrogen atom.

1146. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1144, wherein y of said furanosyl moiety comprises an oxygen atom.

1147. (Canceled)

1148. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said furanosyl moiety comprises a ribose, a deoxyribose or a dideoxyribose.

1149. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said pyrimidine analogs comprise thymidine analogs, uridine analogs, deoxyuridine analogs, cytidine analogs, deoxycytidine analogs or a combination of any of the foregoing.

1150. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1149, wherein said uridine analogs comprise 5-bromo-2'-deoxyuridine-5'-phosphate.

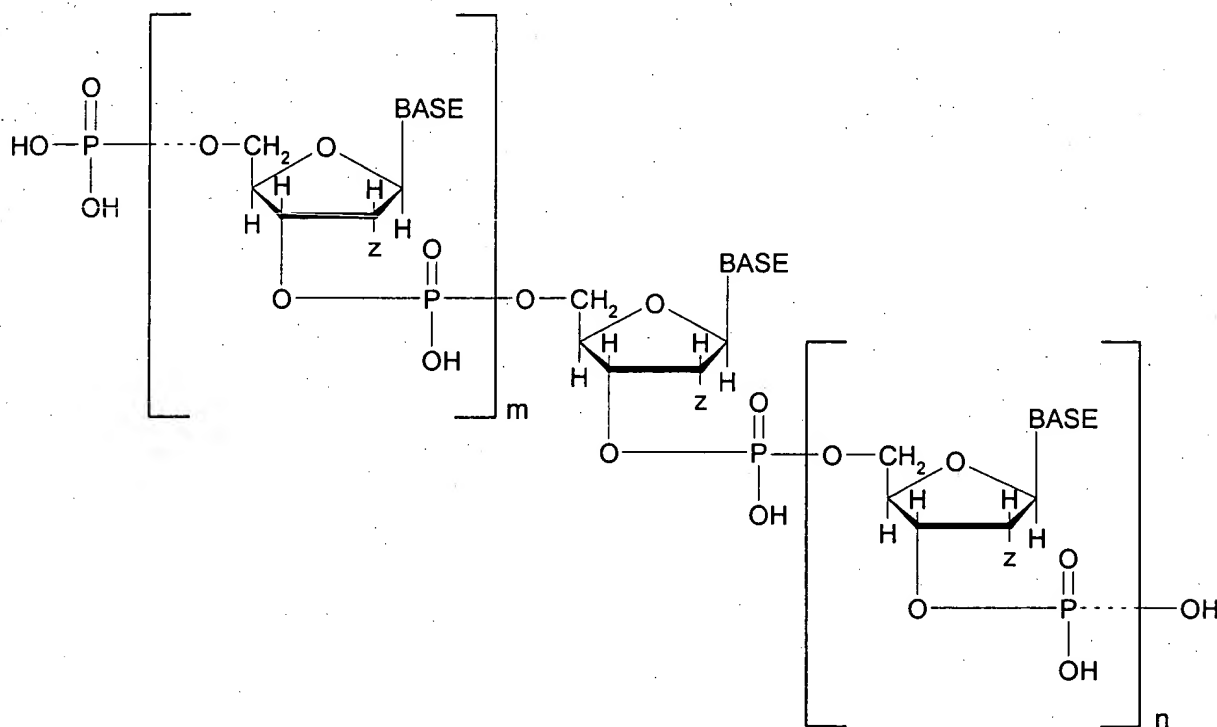
1151. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1149, wherein said deoxycytidine analogs comprise 5-hydroxymethyl-2'-deoxycytidylic acid.

1152. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1121, wherein said purine analogs comprise adenosine analogs, deoxyadenosine analogs, guanosine analogs, deoxyguanosine analogs, or a combination of any of the foregoing.

1153. (Previously Added) The oligo- or polydeoxyribonucleotide of claim 1152, wherein said adenosine analogs comprise tubercidin or toyocamycin.

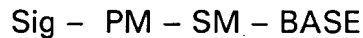
1154. (Currently Amended) An oligo- or polynucleotide comprising the ~~The~~ oligo- or polydeoxyribonucleotide of claim 1121, and further comprising at least one ribonucleotide.

polydeoxyribonucleotide of claim 1121, having the structural formula:



wherein m and n represent integers from 0 up to about 100,000, and wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

1156. (Currently Amended) An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide or modified nucleotide analog having the formula



wherein PM is a phosphate moiety, SM is a furanosyl moiety and BASE is a base moiety comprising a pyrimidine, a pyrimidine analog, a purine, a purine analog, a deazapurine or a deazapurine analog, wherein said analog can be attached to or coupled to or incorporated into DNA or RNA, wherein said analog does not substantially interfere with double helix formation or nucleic acid hybridization, wherein said PM is attached to SM, said BASE is attached to SM, said Sig is covalently attached to PM via a chemical linkage comprising a polypeptide, and wherein Sig comprises a non-polypeptide, non-nucleotidyl, non-radioactive label moiety comprising at least three carbon atoms, and which Sig can be directly or indirectly detected when attached to PM via said polypeptide chemical linkage or when said modified nucleotide or modified nucleotide analog is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide, and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide.

1157. (Canceled)

1158. (Currently Amended) The oligo- or polynucleotide of claim 1156, wherein Sig comprises biotin, iminobiotin, an electron dense component, a magnetic component, a metal-containing component, a fluorescent component, a chemiluminescent component, a chromogenic component, ~~a saccharide component~~ or a combination of any of the foregoing.

1159. (Previously Added) The oligo- or polynucleotide of claim 1158, wherein said magnetic component comprises magnetic oxide.

1160. (Previously Added) The oligo- or polynucleotide of claim 1159, wherein said magnetic oxide comprises ferric oxide.

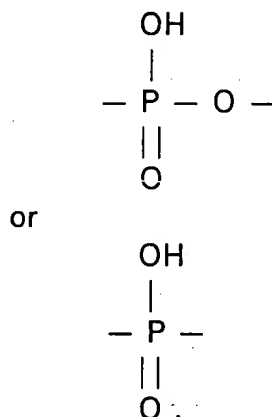
1161. (Previously Added) The oligo- or polynucleotide of claim 1158, wherein said metal-containing component is catalytic.

1162. (Previously Added) The oligo- or polynucleotide of claim 1158, wherein said fluorescent component comprises fluorescein, rhodamine or dansyl.

1163. (Canceled)

1164. (Canceled)

1165. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said covalent attachment comprises:



1166. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said polypeptide chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

1167. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said polypeptide chemical linkage comprises a $-\text{CH}_2\text{NH}-$ moiety.

1168. (Previously Added) The oligo- or polynucleotide of claim 1156, wherein said polypeptide chemical linkage comprises an allylamine group.

Dean L. Engelhardt, et al.
Serial No.: 08/486,069
Filed: June 7, 1995
Page 187 [Amendment Under 37 C.F.R. §1.115 (In Response
To The November 23, 1999 Office Action) - May 23, 2000]

Priority Claim Under 35 U.S.C. §120

As indicated in the opening remarks above, the specification has been amended by inserting on page 2 a new section titled REFERENCE TO OTHER RELATED APPLICATIONS. U.S. Patent Application Serial No. 06/674,352, filed on November 21, 1984 has been cited and its abandoned status has been indicated.

The First Rejection Under 35 U.S.C. §112, First Paragraph

Claim 367 stands rejected for new matter under 35 U.S.C. §112, first paragraph. In the Office Action (page 3), the Examiner stated:

The specific localization of modified nucleotides as given in instant claim 367 has also not been found as filed and is therefore NEW MATTER. It is noted that generic locations of modified nucleotides does not support a specific specie as claimed in claim 367 of specifically two locations never disclosed together in the instant specification.

As indicated in the opening remarks above, the subject matter of former claim 367 has been omitted from the new claims.⁵ Accordingly, the rejection for new matter is believed to have been rendered moot.

In view of the presentation of the new claims, reconsideration and withdrawal of the new matter rejection is respectfully requested.

Before addressing the substantive issues in the November 23, 1999 Office Action, Applicants would like to elaborate on the issue of "nucleotide analogs," "base analogs," "sugar analogs" and "phosphate analogs," as set forth in the new claims. In the Examiner Interview Summary Record dated January 27, 2000, it was indicated that [the Examiner and Applicants' representatives discussed]

⁵ Former and now canceled claim 367 was directed to "[t]he process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one terminal modified nucleotide."

Dean L. Engelhardt, et al.
Serial No.: 08/486,069
Filed: June 7, 1995
Page 188 [Amendment Under 37 C.F.R. 51.115 (In Response
To The November 23, 1999 Office Action) - May 23, 2000]

... "the metes and bounds of nucleotide analogs and self-indicating
in the claim language."

In response, Applicants wish to point out that the new claims are directed to
processes for sequencing, detection and labeling in which modified nucleotides or
nucleotide analogs are utilized in conjunction with non-radioactive labeling and
detection (or radioactive detection in the case where chelating compounds or
components are employed). Nucleotide analogs are defined in the new claims as
those "which can be incorporated into DNA or RNA." An artisan working in the
field of nucleic acids, including nucleic acid sequencing and hybridization detection,
would appreciate and understand the meaning and extent of the foregoing claim
language. Moreover, such an artisan working in the field of nucleic acids would
also appreciate and understand from this claim language which members would be
included in such nucleotide analogs and which members would be excluded.

The term "nucleotide analog" or equivalent language and examples are
disclosed numerous times in the specification.⁶ In fact, there are no fewer than 34
references or examples in the specification to nucleotide analogs and these are
listed below.⁷

Specification References to Nucleotide Analogs

<u>Description</u>	<u>Page/Line</u>
analogs of dUTP and UTP	Page 1, 10th line from bottom
the analogs must be relatively efficient substrates	Page 7, line 9
5-methylcytosine, and 5-hydroxymethylcytosine	Page 9, 2nd & 3rd lines from bottom

⁶ The term "nucleotide analog" naturally embraces nucleotidyl subelements including "sugar
analogs," "phosphate analogs," and "base analogs," as recited in the new claims.
⁷ It should not be overlooked that the disclosure of the original Ward application, U.S. Patent
Application Serial No. 06/225,223, filed on April 17, 1981, was incorporated into the present
specification. It may well be that there are additional instances of references to "nucleotide
analogs" in Serial No. 06/225,223 that have not been included in the 34 references or examples
cited in the present specification and listed here.

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<u>Description</u>	<u>Page/Line</u>
thymidine analog	Page 31, line 14
analogs of dUTP and UTP	Page 37, 12th line from bottom
5-hydroxy-methylcytosine (5 HMC)	Page 54, 2nd and 3rd full paragraphs
reacting nucleic acids in the double helical form with alkylating agents as for example benz(o)pyrene diol epoxide or aflatoxin. Under appropriate conditions the N ² group of guanine, the N ⁶ group of adenosine or the N ⁴ group of cytosine are alkylated	Page 54, last paragraph
5-Hydroxymethyl-2'-deoxycytidylic acid	Page 60, Example X
5-(4-aminobutylaminomethyl)-2'-deoxyuridylic acid	Page 61, Example XI
Biotinylated-5-(4-aminobutylaminomethyl)-2'-deoxy- uridylic acid	Page 61, Example XII
5-formyl-2'-deoxyuridine	Page 62, Example XIII
Biotinylated 5-formyl-2'-deoxyuridine	Page 63, Example XIV
Biotinylated 5-amino-2'-deoxyuridine	Page 63, Example XV
5-(oxy)acetic acid-2'-deoxyuridine	Page 64, Example XVI
Biotinylated 5-(oxy)acetic acid-2'-deoxyuridine	Page 64, Example XVII
5-hydroxymethyl-2'-deoxycytidine-5'-triphosphate	Page 66, Example XIX
Enz-5(D8)(C2)	

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<u>Description</u>	<u>Page/Line</u>
maltotriose nucleotide [maltotriose coupled 5-(3-amino-1-propenyl)-2'-deoxyuridine-5' triphosphate	Page 71, Example XXIII
5-(perfluorobutyl)-2'-deoxyuridine	Page 72, Example XXIV
Tubercydin	Page 72, Example XXV
Toyocamycin	Page 72, Example XXV
Maltotriose coupled 5-(3-amino-1-propenyl)-2'-deoxy-uridine-5' triphosphate	Page 75, Example XXXI
Fluorescein coupled 5-(3-amino-1-propyl)-2'-deoxy-uridine-5'-triphosphate (AA-dUTP)	Page 76, Example XXXII
5-Bromo-2'-deoxyuridine-5'-phosphate	Page 78, Example XXXV
6-Cyano-2'-deoxyuridine-5'-phosphate	Page 79, Example XXXVII
6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid	Page 80, Example XXXVIII
Two minor purines	Page 91
2-Methyladenine	
1-Methylguanine	
Two minor pyrimidines	Page 91
5-Methylcytosine	
5-Hydroxymethylcytosine	

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<u>Description</u>	<u>Page/Line</u>
A nucleotide . . . wherein said base B is 2-methyladenine	Original Claim 71
A nucleotide . . . wherein said base B is 1-methylguanine	Original Claim 71
A nucleotide . . . wherein said base B is 5-methylcytosine	Original Claim 72
A nucleotide . . . wherein said base B is 5-hydroxymethylcytosine	Original Claim 73
A nucleotide . . . wherein said base B is deazaadenine	Original Claim 75
A nucleotide . . . wherein said base B is deazaguanine	Original Claim 76

The specification also describes numerous instances for the attachment,
coupling or incorporation of modified nucleotides or nucleotide analogs into DNA or
RNA. These instances are set forth below.

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Specification References to Attachment, Coupling and Incorporation

<u>Description</u>	<u>Page/Line</u>
nucleotides are modified, such as at the 5 position of pyrimidine or the 7 position of purine, preparatory for the preparation for the preparation therefrom of nucleotide probes suitable for attachment to or incorporation into DNA or other nucleic acid material	Page 52, last paragraph (under "Summary of the Invention")
Oligodeoxyribonucleotides were end-labeled using cytidine-5'-triphosphate and terminal transferase	Page 56, Example IV
Biotin and polybiotinylated poly-L-lysine were coupled to oligoribonucleotides	Page 57, Example V
Formaldehyde coupling of cytochrome C-biotin and polybiotinylated poly-L-lysine to oligodeoxyribonucleotides were carried out	Page 58, Example VII
Ligation of poly dA:poly dT, biotinyl dU to oligodeoxyribonucleotides was accomplished	Page 60, Example IX
labeling purified DNA [by nick translating] with biotinylated 5-formyl-2'-deoxyuridine	Page 67, Example XX
Lambda DNA was nick translated as described herein with maltotriose coupled to 5-(3-amino-1-propenyl)-2'-deoxyuridine-5' triphosphate and 3H-2'-deoxyadenosine	Page 71, Example XXIII
A DNA probe was ligated to a synthetic DNA composed of repeated sequences of <i>E. coli</i> lac operator DNA	Page 77, Example XXXIV

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<u>Description</u>	<u>Page/Line</u>
As indicated hereinabove, various techniques may be employed in the practices of this invention for the incorporation of the special nucleotides of this invention into DNA and related structures. One particularly technique referred to herein involves the utilization of terminal transferase for the addition of biotinates dUMP onto the 3' ends of a polypyrimidine or to single-stranded DNA. The resulting product, such as a single-stranded or cloned DNA, which has biotinates dUMP onto the 3' ends thereof, can be recovered . . .	Page 99, second paragraph
These nucleotides are then incorporated into specific nucleic acids using a DNA or RNA polymerase or ligase reaction or a chemical linkage.	Page 101, first paragraph (third sentence)
A nucleotide in accordance with Claim 1 wherein . . . such that when said nucleotide is incorporated into or attached to or associated with a double-stranded deoxyribonucleic acid or double-stranded ribonucleic acid or DNA-RNA hybrid, . . .	Original claim 7
A ribonucleotide in accordance with Claim 143 wherein when said nucleotide is incorporated into or attached to a double-stranded deoxyribonucleic acid or double-stranded ribonucleic acid or DNA-RNA hybrid, . . .	Original claim 145

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With specific reference to "nucleotide analog which can be incorporated into DNA or RNA," Applicants also wish to point out that this term was well-known and recognized in the art before the advent of their first patent application filing in June 1982.

The literature, which includes an entire textbook devoted specifically to nucleotide analogs (and actually titled "Nucleotide Analogs") as well as other well-known textbooks by the Nobel Prize winning author and scientist, Dr. Arthur Kornberg of Stanford University, is replete with references to nucleic analogs and more particularly, nucleotide analogs which can be attached to or coupled to or incorporated into DNA or RNA or other nucleic acid or genetic structures or material.

In his 1980 textbook titled Nucleotide Analogs: Synthesis and Biological Function [John Wiley & Sons, Inc., New York, 1980, 288 pages], Professor Dr. Karl Heinz Scheit of the Max-Planck-Institut für Biophysikalische Chemie in Göttingen, Germany, provided an exquisite account of nucleotide analogs covering almost 300 pages. The nucleotide analog compounds described by Dr. Scheit include nucleotides with modified heterocyclic substituents (base analogs), nucleotides with modified phosphate groups (phosphate analogs) and nucleotides with altered sugar parts (sugar analogs). The subject of nucleotides with modified heterocyclic substituents is covered by Dr. Scheit in Chapter 2 (pages 13-89) while the subjects of nucleotides with modified phosphate groups and altered sugar parts are described in Chapter 4 (pages 96-141) and Chapter 5 (pages 142-194), respectively. A complete copy of Dr. Scheit's book is being submitted as Exhibit 1 in Applicants' Supplemental Information Disclosure Statement concurrently filed with their Amendment.

In his 1974 textbook on DNA Synthesis [W. H. Freeman And Company, San Francisco, Chapter 7, pages 227-228, copy attached to Supplemental IDS as Exhibit 2], Dr. Kornberg described several nucleotide analogs which are incorporated into DNA, RNA or DNA and RNA. These include the following:

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Dideoxynucleoside triphosphates	Incorporated into DNA
Arabinosyl nucleoside triphosphates	Incorporated into DNA
Cordycepin triphosphates (3'-deoxy ATP)	Incorporated into DNA and RNA
3'-Amino ATP	Incorporated into DNA and RNA
dUTP	Incorporated into DNA
5-Hydroxyuridine, or 5-aminouridine	Incorporated into RNA
5-Bromouracil	Incorporated into DNA
Tubericidin "ATP", Formycin "ATP"	Incorporated into RNA and DNA
Dideoxynucleoside triphosphates	Incorporated into DNA
Arabinosyl nucleoside triphosphates	Incorporated into DNA
Cordycepin triphosphates (3'-deoxy ATP)	Incorporated into DNA and RNA
3'-Amino ATP	Incorporated into DNA and RNA
dUTP	Incorporated into DNA
5-Hydroxyuridine, or 5-aminouridine	Incorporated into RNA
5-Bromouracil	Incorporated into DNA
Tubericidin "ATP", Formycin "ATP"	Incorporated into RNA and DNA

In a later textbook DNA Replication published in 1980 (W. H. Freeman And Company, San Francisco, Chapter 12, "Inhibitors of Replication," pages 415-441; copy attached to Supplemental IDS as Exhibit 3), Dr. Kornberg devotes an entire chapter subsection to the subject of nucleotide analogs incorporated into DNA or RNA. In fact, the very title of the Subsection 12-3 beginning on page 423 in Dr. Kornberg's book is "Nucleotide Analogs Incorporated into DNA or RNA." The subsection begins with

Certain analogs of the nucleoside triphosphates, modified in the sugar or base, are accepted by polymerases for pairing with the DNA template and are incorporated into nucleic acid. . .

On page 423 in DNA Replication, Dr. Kornberg then proceeds to list no less than 16 different nucleotide analogs incorporated into DNA or RNA, including the following:

Enz-5(D8)(C2)

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2',3'-Dideoxy NTPs	5-Iodouracil
Arabinosyl NTPs (araC, araA)	Tubercidin
Cordycepin TP (3'-deoxy ATP)	Toyocamycin
3-Amino ATP	Formycin
Uracil dNTP (dUTP)	7-Deazanebularin
5-Hydroxyuridine	2-Aminopurine
5-Aminouridine	2-Aminoadenine (2,6- diaminopurine)
5-Bromouracil	2'-Deoxy,2'-azidocytidine

On the next page (224) in Figure 12-4 which is titled "Nucleotide analogs incorporated into DNA or RNA," Dr. Kornberg lists examples of sugar analogs, base analogs of uridine and thymidine, base analogs of adenine and base analogs of adenosine, including the 11 analogs listed below:

Sugar analogs

2',3'-Dideoxynucleoside
Arabinosynucleoside
Cordycepin
3'-Aminoadenosine

Base analogs of uridine & thymidine

5-Hydroxy-, 5-Amino-, 5-Bromo-, 5-Iodouracil
5-Hydroxy-, 5-Amino-, 5-Bromo-, 5-Iodothymidine

Base analogs of adenine

2-Aminopurine
2-Aminoadenine

Base analogs of adenosine

Tubercidin
Formycin
Toyocamycin
7-Deazanebularin

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Other notable textbooks published before the 1982 filing date of this application have also disclosed nucleotide analogs which can be incorporated into RNA and DNA. By way of example, these include the three books described further below.

Dr. J. N. Davidson of the University of Glasgow, Edinburgh, Scotland, published seven editions of The Biochemistry of the Nucleic Acids before his death in 1972. Four of his colleagues in the Department of Biochemistry, University of Glasgow, authored an eighth edition published in 1976 and appropriately titled Davidson's The Biochemistry of the Nucleic Acids (8th Edition) (Revised by R. L. P. Adams, R. H. Burdon, A. M. Campbell and R. M. S. Smellie, Academic Press, New York, 1976, copy attached to Supplemental IDS as Exhibit 4).⁸

On pages 298 and 299 of Chapter 11 "Replication of DNA," the authors describe analogs in Subsection 11.7.1 *Base and nucleoside analogues*:

Some of the artificially produced base analogues are incorporated into RNA and DNA and may have powerful mutagenic effects [296, 301, 302, 304, 308, 309]. Among the most important analogues are the halogenated pyrimidines, and those bases where nitrogen has been substituted for a —CH= group (see Fig. 11.29).

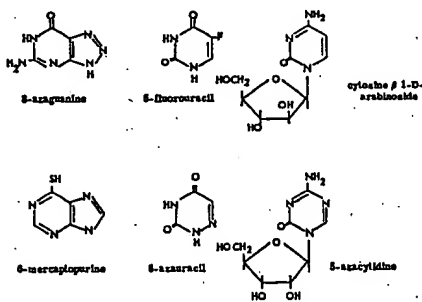


Fig. 11.29 Structures of some purine and pyrimidine analogues

⁸ Later editions of this work by Adams' group also disclosed the same material on base and nucleoside analogues, including their Tenth Edition published in 1986. Although not reviewed by Applicants' attorney in the course of preparing this Declaration, one would expect that the Ninth Edition of The Biochemistry of the Nucleic Acids published in 1981 and one year before the filing date of this patent application would contain similar if not identical subject matter on base and nucleoside analogues as in the Eighth and Tenth Editions.

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On page 299, a second action of these base and nucleoside analogs is described:

(2) They [base and nucleoside analogs] are themselves, after conversion into nucleotides, incorporated to varying degrees into RNA and/or DNA, although the incorporation may take an abnormal form. Thus 8-azaguanine can be incorporated at the expense of guanine into the RNA of TMV [317] and, to a much larger extent, into the RNA of *B. cereus* [citation omitted]. Only very small amounts are incorporated into the DNA.

5-Azacytidine is incorporated into RNA but this rapidly interferes with protein synthesis [162, 304]. 5-Azadeoxycytidine is incorporated into DNA, but this renders the cells non-viable [319]. 5-Bromouracil can replace thymine in DNA where it normally base-pairs with adenine. However, in its rare enol-state (which it assumes more readily than does thymine) it may pair with guanine instead of adenine so bringing about the base-pair transition of A-T into G-C. DNA containing 5-bromouracil instead of thymine is very susceptible to breakage at light-induced bromouracil dimers [320] (see below).

The D-arabinosyl nucleosides are effectively analogues of deoxyribonucleosides (e.g. cytosine β -D-arabinoside is incorporated in place of deoxycytidine into DNA where it causes chain termination or a marked reduction in the rate of further chain extension [320-322].

Another significant text disclosing nucleotide analogs and published a year earlier in its English translation earlier than Davidson's Biochemistry of the Nucleic Acids was written by Peter Langen [Antimetabolites of Nucleic Acid Metabolism: The Biochemical Basis of Their Action, with Special Reference to their Application in Cancer Therapy, Gordon and Breach, New York, English edition translated from the German by Dr. Thomas A. Scott, 1975, pages 143-187, copy attached to Supplemental IDS as Exhibit 5]. The second section of Langen's book covers more than twenty-five pages and is devoted to pyrimidine analogs and their nucleosides, purine analogs and their nucleosides, nucleoside analogs with modified sugar components and nucleotide analogs with modified phosphate groups. Almost 70 such analogs are described by Langen, many of which are incorporated into DNA or RNA, as set forth in the quoted passages below.

(1)

2. 5-Fluorouridine 5'-phosphate is incorporated into RNA in place of uridine 5'-phosphate. This incorporation and its biological consequences are discussed in detail on page 71.

[page 144]

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(2)

The preparation and the elucidation of the mechanism of action of 5-trifluoromethyl-2'-deoxyuridine are due to HEIDELBERGER et al. It is incorporated into DNA in place of thymidine.

[page 146]

(3)

Owing to the similarity in the atomic radii of bromine (1.95 Å), iodine (2.16 Å) and the methyl group (2.00 Å), 5-bromo- and 5-iodo-2'-deoxyuridine are metabolized by the cell in the same way as thymidine and incorporated into DNA.

[page 147]

(4)

Up to 100% of the thymine of DNA can be replaced by analogues.

[page 147]

(5)

In normal tissues with a high rate of mitosis, e.g. bone marrow and intestinal epithelium, the rate of incorporation of halogenated thymine analogues into DNA is higher than in most types of tumour.

[page 148]

(6)

5-Chlorouracil, like 5-bromouracil and 5-iodouracil, was recognized very early as an inhibitor of bacterial growth [773; 775], but has not been extensively studied. Considering the size of the atomic radius of chlorine, it should be possible to incorporate 5-chlorouracil into both DNA and RNA (see p. 6).

[page 148]

(7)

5-Aminouracil is incorporated into DNA [876] and into RNA [1681].

[page 149]

(8)

5-Methylamino-2'-deoxyuridine causes a transitory increase in the growth of thymine auxotrophs of *E. coli*, which is followed by cell death [1807]. It is possibly incorporated into DNA.

[page 149]

(9)

The activity of 5-hydroxyuracil is similar to that of 5-aminouracil and its inhibitory action on bacterial growth was discovered very early [773; 775]. The preparation and use of the riboside and deoxyriboside was reported later [97]. The riboside is phosphorylated in the cell to the triphosphate, which inhibits RNA-polymerase and is also incorporated into RNA [1491; 1682].

[page 151]

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(10)

5-Ethyluracil is incorporated into the DNA of thymine auxotrophs of *E. coli* and of T3 phage cultured on these strains (see p. 70). Since the base is a poor substrate for thymidine phosphorylase [1361], a much higher incorporation is obtained after applying the 2'-deoxyriboside.
[page 151]

(11)

The cytostatic and virostatic activity of 2-thiouracil are due to its incorporation into RNA (see p. 87).
[page 154]

(12)

To a limited extent, the 5'-triphosphate of 4-thiothymidine can replace thymidine 5'-triphosphate in the *E. coli* polymerase system. After the attachment of a few residues of 4-thiothymidine 5'-triphosphate, the polymer primer is effectively "poisoned" and the reaction stops.
[page 154]

(13)

5-Azacytidine is incorporated into DNA and RNA and thereby disturbs the function of the nucleic acids (see pp. 71 and 90).
[page 158]

(14)

6-Azathymine was discovered very early as an inhibitor of bacterial growth [453; 1403; 1404; 1415]. Although it is incorporated into DNA in place of thymine (see p. 71), this is not responsible for the inhibition of growth [1403; 1404].
[page 159]

(15)

2-Fluoroadenosine 5'-triphosphate inhibits RNA-polymerase and, at the same time, some of the analogue is possibly incorporated into RNA [1611].
[page 167]

(16)

2-Aminopurine is incorporated into DNA. The incorporation and its consequences are discussed more fully on page 72. . . The 5'-triphosphate, which is necessary for the incorporation into DNA, is formed by the phosphorylation of 2'-deoxyriboside.
[page 167]

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(17)

Tubercidin is an antibiotic from *Streptomyces tubercidicus*. It is converted intracellularly into the mono-, di- and triphosphates [6; 397] and incorporated into the DNA and RNA (see p. 89). Tubercidin 5'-di (or tri) phosphate is a substrate for ribonucleotide reductase [1730], as would be expected from its incorporation into DNA.

[page 170]

(18)

Toyocamycin is an antibiotic from *Streptomyces*. It is phosphorylated intracellularly to the 5'-mono, di- and triphosphates, and incorporated into DNA and RNA [1731] (see p. 89). As in the case of tubercidin, the 5'-di, or 5'-triphosphate is a substrate for ribonucleotide reductase, thus fulfilling the requirement for incorporation into DNA [1730].

The incorporation of this compound into nucleic acids and polynucleotides is discussed more fully on page 89. . .

[pages 170 & 171]

(19)

8-Azaguanine was known as early as 1945 as a growth inhibitor of *E. coli* [1470]. Investigations by KIDDER et al [737; 946] with *Tetrahymena gelii* and by SKIPPER et al [1236] with tumours showed that the antimetabolite is incorporated into nucleic acids. This was the first observation of the incorporation of an unnatural base into nucleic acids.

. . . The consequences of the incorporation of 8-azaguanine into RNA are discussed more fully on page 86. The incorporation of 8-azaguanine into DNA is either very low or non-existent (e.g. [1684]. . .

[page 171]

(20)

3-Deoxyadenosine (cordycepin) is an antibiotic from *Cordyceps militaris*. It is phosphorylated intracellularly to the mono-, di- and triphosphates [975; 976; 1610] and is incorporated into DNA [353] and RNA [1609]. The inhibition of RNA-polymerase by 3'-deoxyadenosine [526; 977; 1614] is due to the incorporation of the inhibitor into the chain ends of RNA; further extension of the chain is then prevented by the absence of the 3'-hydroxyl group.

[page 176]

(21)

3'-Deoxy-3'-aminoadenosine, which is also an antibiotic from *Cordyceps militaris*, inhibits RNA-polymerase and is possibly incorporated into RNA [1610; 1784].

[page 176]

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(22)
2',3'-Dideoxyadenosine inhibits the multiplication of *E. coli* [1213].
The inhibition appears to be due to the incorporation of inhibitor into
DNA (see p. 72), which prevents further extension of the chain.
[page 177]

(23)
3'-Deoxythymidine may also be included here. This compound is not
itself biologically active, because it is not phosphorylated in the cell.
The chemically prepared 5'-triphosphate is incorporated into DNA in
the cell free system, where it inhibits further chain extension.
[page 178]

(24)
L-Nucleosides are taken up by mammalian cells, but not by bacteria
[1815a]. L-Adenosine is phosphorylated in the mammalian cell to the
5'-monophosphate and, to a much less extent, to the 5'-diphosphate
[873]. It is probably also incorporated into RNA.
[page 183]

(25)
... Adenosine 5'-(methylenediphosphonyl) phosphate can replace
adenosine 5'-triphosphate in the isolated RNA-polymerase system
[1635]. ...
... In some reactions, e.g. the formation of the complex that
initiates peptide elongation on the ribosome, guanosine 5'-triphosphate
can be replaced by its analogue [Guanosine 5'-
(methylenediphosphonyl) phosphate], but the subsequent reactions,
which involve the decomposition of the guanosine 5'-triphosphate, are
inhibited.
[page 187]

Many scientific articles have also been published on the subject of nucleotide
analogs, including those which are incorporated into DNA or RNA. By way of
examples, the eight (8) articles listed below, all of which were published before
1982, disclose such nucleotide analogs as those recited in the claims of this patent
application. Included below are brief quotations from these articles of references or
examples of nucleotide analogs which can be incorporated into DNA or RNA.

A. Darlix et al., "Analysis of Transcription *in Vitro* Using Purine Nucleotide
Analogues," Biochemistry 10:1525-1531. (1971) [copy attached to Supplemental IDS
as Exhibit 6].

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(1)

Formycin and 8-deazaguanine, structural analogs of adenosine and guanosine, respectively (Robin *et al.* (1945); Koyana *et al.* (1966), inhibit nucleic acid synthesis in bacterial and mammalian cells (Shapiro *et al.* (1950); Chantrenne (1964); Hori *et al.* (1964); Ward *et al.* (1969)). The analogs resemble their normal counterparts in H-bonding potential and in the formation of base pairs with complementary template residues, and they are also effectively incorporated into RNA *in vivo* and *in vitro* (Shapiro *et al.* (1950); Smith and Mathews (1957); Mathews (1957); Brockman *et al.* (1959); Caldwell *et al.* (1966); Ward *et al.* (1969).

[page 1525, left column, 1st ¶]

(2)

... Under these conditions the relative yield of chains initiated by the purine nucleotide analogs [8-azaGTP and FTP] was decreased still further.

[page 1526, right column, penultimate ¶]

(3)

(c) INITIATION KINETICS AT LOW NUCLEOTIDE ANALOG CONCENTRATIONS.

[page 1527, right column, last ¶]

(4)

III. Effect of Purine Analogs on Chain Release.

[page 1530, left column, 1st full ¶]

(5)

Discussion

The results presented in this paper provide additional insights into possible biochemical mechanisms of action of base analogs and identify some steps in transcription of DNA which may be susceptible to exogenous controls.

Our data show that individual stages in transcription can be differentiated by their response to the base analogs; ...

[page 1530, left column, last two ¶s]

B. Geider, K., "DNA Synthesis in Nucleotide-Permeable *Escherichia coli* Cells: The Effects of Nucleotide Analogues on DNA Synthesis," European Journal of Biochemistry 27:554-563 (1972) [copy attached to Supplemental IDS as Exhibit 7].

(1)

The effects of nucleotide analogues on DNA synthesis were studied in nucleotide-permeable *Escherichia coli* cells.

[page 554, abstract]

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(2)

RESULTS

Inhibition of DNA Synthesis by 22'.3'-Dideoxyribosylthymine Triphosphate

Log phase *E. coli* H512 was harvested and made permeable to nucleotides by shaking the 100-fold concentrated cell suspension with ether as described in Materials and Methods. DNA synthesis in the presence of deoxyribonucleoside triphosphate is strongly inhibited by ddTTP, a dTTP analogue lacking the 3'-hydroxyl group. Increasing concentrations of ddTTP reduce the deoxyribonucleoside triphosphate incorporation into *E. coli* DNA more and more (Fig. 1, lower curves) . .

[page 556, left column, 1st full ¶]

C. Darlix and Fromageot, "Restriction of gene transcription by nucleotide analogs," *Biochimie* 56:703-710 (1974) [copy attached to Supplemental IDS as Exhibit 8].

(1)

In this communication, the influence of the purine nucleoside triphosphate analogs on RNA chain termination induced by rho was investigated, using T7 phage DNA as a template . . .

[page 703, left column, 1st ¶]

(2)

RESULTS

EFFECTS OF PURINE ANALOGS ON *in vitro* T7 DNA TRANSCRIPTION.

[page 703, lower right column]

(3)

. . . So both purine analogs tend to restrict gene transcription in the presence of rho, albeit FTP less dramatically than 8-azaGTP.

[page 706, lower left column]

(4)

. . . Double stranded(sic) ribopolymers containing either analog were synthesized with *E. coli* RNA polymerase to directly test the influence of these purine analogs on RNase III activity . . .

[page 706, right column, 2nd ¶]

(5)

Finally, the restriction of gene transcription by nucleotide analog might be exploited to achieve selective expression of particular regions of genomes *in vivo* and/or *in vitro*.

[page 709, right column, last ¶]

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D. Marcus, F., "Inhibition of Fructose 1,6-Biphosphatase by 9-β-D-Arabinofuranosyl 5'-Monophosphate," Cancer Research 36:1847 (1976) [copy attached to Supplemental IDS as Exhibit 9].

(1)

There has been an increased interest in the study of nucleoside analogs as potential therapeutic agents in the treatment of neoplastic diseases (for references, see Ref. 1) The field is also expanding to nucleotide analogs . . .

[page 1847, 1st ¶]

(2)

Other close AMP [adenosine monophosphate] analogs (i.e. the 5'-phosphates of deazaadenosine, formycin, isoadenosine, tubercidin) may also inhibit fructose 1,6-bisphosphatase, thereby altering the regulation of carbohydrate metabolism. Adenine nucleotide analogs could also affect the key regulatory glycolytic enzyme phosphofructokinase, as recently mentioned by Bloch³ in his studies with tubercidin.

[page 1847, 2nd ¶]

E. Simoncsits and Tomász, "A New Type of Nucleoside 5'-Triphosphate Analogue: P1-(Nucleoside 5'-) P1-Amino-Triphosphates," Tetrahedron Letters 44:3995-3998 (1976) [copy attached to Supplemental IDS as Exhibit 10].

Compounds of type 2 as nucleoside 5'-triphosphate analogues containing chemically modified, chiral α-phosphorus atoms may be important from the point of view of different chemical and enzymatic studies . . .

[page 3997, last ¶]

F. Chladek et al., "Synthesis and Properties of Nucleoside 5'-Phosphoazidates Derived from Guanosine and Adenosine Nucleotides: Effect on Elongation Factors G and Tu Dependent Reactions," Biochemistry 16:4312-4319 (1977) [copy attached to Supplemental IDS as Exhibit 11].

(1)

ABSTRACT: A new type of nucleoside poly(5'-phosphate) analogue, nucleoside 5'-phosphoazidate, with an azido group on the terminal phosphate of GTP, ATP, GDP, GMP, and AMP, has been synthesized . . .

[page 4312, top of the page]

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(2)

We have chosen as a probe the photolabile azido group, placed at the terminal phosphate of GTP and GDP. In this paper, we describe our initial studies of the synthesis and properties of these new compounds, the nucleoside 5'-phosphoazidates. We also report on the behavior of these analogues of GTP and GDP . . .

[page 4312, right column, 2nd full ¶]

(3)

Results

Synthesis and Proof of Structure of Nucleoside 5'-Phosphoazidates IIIa-f. Michelson (1964) reported the synthesis of various nucleoside anhydrides as well as nucleotide anhydrides by anion displacement on p1-nucleoside 5'-p2-diphenylpyrophosphates. It was found that a variety of anions of acids weaker than diphenylphosphoric acid could attack esterified pyrophosphates of the general type II (Scheme II) . . . We have used the method of Michelson (1964) for the synthesis of the novel nucleotide analogues, nucleoside 5'-phosphoazidates (IIIa-f).

[page 4315, lower right column, through page 4316, 1st ¶]

G. Piperno and Alberts, "An ATP Stimulation of T4 DNA Polymerase Mediated via T4 Gene 44/62 and 45 Proteins," Journal of Biological Chemistry **253**:5174-5179 (1978) [copy attached to Supplemental IDS as Exhibit 12].

(1)

. . . In this report we use nucleotide analogues to demonstrate that this polymerase stimulation requires hydrolysis of the β,γ -phosphate bond of ATP.

[page 5174, abstract]

(2)

In this study we use rATP and dATP analogues to demonstrate that the above stimulation of T4 DNA polymerase by the accessory proteins requires the hydrolysis of ATP or dATP . . .

[page 5174, right column, last line, through
page 5175, left column, first two lines]

H. Reha-Krantz et al., "Bacteriophage T4 DNA Polymerase Mutations That Confer Sensitivity to the PPi Analog Phosphonoacetic Acid," Journal of Virology **67**:60-66 (1993) [copy attached to Supplemental IDS as Exhibit 13].

(1)

. . . As found for herpes simplex virus DNA polymerase, T4 mutations that altered sensitivity to phosphonoacetic acid also altered sensitivity to nucleotide analogs . . .

[page 60, abstract]

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(2)

. . . As was observed for HSV DNA polymerase studies, T4 DNA polymerase mutations that altered PAA sensitivity also affected interactions with nucleotide analogs; . . .

[page 60, left column, last ¶]

(3)

Sensitivity to ddNTPs and other nucleotide analogs. HSV DNA polymerase mutant strains isolated for the ability to replicate in the presence of PAA or PFA are often less sensitive to the antiviral action of nucleotide analogs such as acyclovir (16, 27) and are cross resistant to 2',3'-dideoxynucleoside triphosphates (ddNTPs) (9)

[page 63, right column, 1st full ¶]

(4)

. . . but it is predicted from HSV DNA polymerase studies that altered sensitivities to nucleotide analogs will also be detected for at least some of the mutants.

[page 63, right column, 2nd full ¶]

(5)

Mutant DNA polymerase respond in a consistent pattern to PPi and nucleotide analogs. As discussed above, wild-type HSV DNA polymerase is sensitive to PAA and mutants that are PAA sensitive are cross resistant to the nucleotide analogs acyclovir and ddNTPs but hypersensitive to PAA (44) . . . While wild-type T4 DNA polymerase is highly resistant to PAA, mutants that are PAA sensitive are cross resistant to nucleotide analogs.

[page 63, right column, last line, through page 64,
left column, 1st two lines]

(6)

. . . Derse et al. (9) suggested that these conflicting observations can be explained if PAA and nucleotide analogs have similar inhibitory mechanisms: . . . If either of these models is correct, it is predicted that DNA polymerase mutations that alter PPi and nucleotide analog interactions would also alter translocation . . .

[page 64, left column, 1st full ¶]

(7)

Identification of a DNA polymerase active center. Single amino acid substitutions at several sites in T4 DNA polymerase and in other family B DNA polymerases altered interactions with both PPi and nucleotide analogs, . . . Gibbs et al. (16) proposed from their studies of HSV DNA polymerase that none of the regions identified in the drug sensitivity studies is the sole binding site for either PPi or dNTPs because amino acid substitutions that alter PAA or PFA sensitivity also alter interactions with nucleotide analogs and/or aphidicolin.

[page 65, left column, 1st full ¶]

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Sugar Analogs

Scientific articles have also been published on the subject of sugar analogs, including those sugar analogs which can be incorporated into DNA or RNA. Listed below are five (5) articles including brief descriptions or references to the term "sugar analog."

A. Birch and Lee, "Structural Functions and Taste in the Sugar Series: The Structural Basis of Bitterness in Sugar Analogues," Journal of Food Science 41:1403-1407 (1976) [copy attached to Supplemental IDS as Exhibit 14].

(1)

... This report re-examines some of the published information relating to sweetness and bitterness in sugars and their analogues in an attempt to explore molecular characteristics which may be responsible for eliciting the bitter response.

[page 1403, left column, 1st ¶]

(2)

MATERIALS & METHODS

ALL SUGARS and their analogues described or referred to in this report were either chromatographically pure crystalline materials obtained from British Drug Houses (Chemicals, Poole, Dorset) or known and novel compounds synthesized by classical carbohydrate techniques as previously reported.

[page 1403, left column]

(3)

... With the sugars and analogues, panelists were asked to place a few milligrams of each substance on the tongue and to comment whether ...

[page 1403, left column, last three lines]

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(4)

RESULTS & DISCUSSION

CONSIDERABLE NUMBERS of the sugar, model glycosides and analogues have already been examined . . .

[page 1403, right column]

B. Larrey and Derechin, "Preparation and Study of a Fluorescent Sugar Analog: Competitive Inhibitor of Yeast Hexokinase," Preparative Biochemistry 9:85-95 (1979) [copy attached to Supplemental IDS as Exhibit 15].

. . . this investigation may open a new direction for active center research since a number of additional fluorescent sugar analogs, each exhibiting a distinct binding property, can be synthesized using similar procedures to those employed here. The potential usefulness of such compounds . . . follows from the known facts . . . and (ii) each subunit in the dimer carries one sugar binding site which, depending on experimental conditions, will bind one sugar (or sugar analogue) or another up to a maximum of two such ligands per dimer¹⁷.

[page 93, 2nd ¶]

C. Roberts and Hayes, "Effects of 2-deoxy D-glucose and other sugar analogues on acid production from sugars by human dental plaque bacteria," Scandinavian Journal of Dental Research 88:201-209 (1980) [copy attached to Supplemental IDS as Exhibit 16].

(1)

Key words: acid production; dental plaque; sugar; sugar analogues.
[page 801, under the abstract]

(2)

In the present study, rates of acid production were measured using aliquots of the same plaque suspension so as to compare acid formation from a range of sugars and sugar alcohols and to investigate the usefulness of 2DG and other sugar analogues as inhibitors of acid production . . .

[page 801, right column]

D. Kortnyk et al., "CMP and CMP-sugar analogs as inhibitors of sialic acid incorporation and glycoconjugates," Eur. J. Med. Chem. - Chimica Therapeutica 15:77-84 (1980) [copy attached to Supplemental IDS as Exhibit 17].

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CMP and CMP-sugar analogs have been synthesized and evaluated as inhibitors of CMP-N-acetylneuraminic acid : glycoprotein sialyltransferase (E.C. 2.4.99) as well as L 1210 murine leukemia cells in culture and *in vivo* as anticancer agents . . .

[page 77, abstract]

E. Keppler et al., "Uridylate trapping, induction of UTP deficiency, and stimulation of pyrimidine synthesis *de novo* by D-galactosone," Biochemical Journal **206**:139-146 (1982) [copy attached to Supplemental IDS as Exhibit 18].

(1)

D-galactosone (D-lyxo-2-hexosulose) was among the first sugar analogues synthesized (Fischer, 1889). In contrast to D-glucosone, this C-2 modified D-galactose analogue was previously not considered to be toxic (Bayne, 1952; Bayne & Fewster, 1956). Earlier work has established that the toxicity of some sugar analogues, including D-galactosamine, is due to their interference with uracil nucleotide . . .

[page 139, left column]

(2)

Our present investigation with a D-galactose-metabolizing hepatoma cell line (Keppler, 1974a) and in rat liver have revealed that D-galactosone can act as a most powerful uridylate-trapping agent, inducing a higher rate of pyrimidine synthesis *de novo* than any of the sugar analogues studied previously . . .

[page 139, right column, last ¶, through page 140, first three lines]

(3)

Discussion

D-galactosone acts as a powerful uridylate-trapping sugar analogue in hepatoma cells and liver . . .

[page 145, left column]

(4)

The interference of uracil nucleotide metabolism induced by D-galactosone indicates that this C-2-modified hexose analogue can be highly effective in D-galactose-metabolizing tissues and cells such as liver and hepatoma . . . This property may be most useful in experimental chemotherapy where uridylate-trapping hexose analogues serve to induce short-term UTP deficiency . . .

[page 146, left column, last paragraph]

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Phosphate Analog (or equivalent language)

The term "phosphate analog" or equivalent language also appeared in the scientific literature before the June 1982 filing date of the present application. The two articles listed below both refer to "phosphate analog" or equivalent language.

A. Yang and Metzler, "Pyridoxal 5'-Phosphate and Analogs as Probes of Coenzyme-Protein Interaction," Methods in Enzymology 62:528-551 (1979) [copy attached to Supplemental IDS as Exhibit 19].

(1)

Reconstitution of Apoenzymes with Pyridoxal-P, Pyridoxamine-P, and Analogs

A variety of derivatives and analogs of the coenzymes are available. The coenzymes may be methylated on the ring nitrogen, on phenolic oxygen, or on the phosphate group.

[page 540, bottom of page, through page 541, 1st line]

(2)

... For example, from Fig. 2 we conclude that the 430-nm substrate aldimine is more strongly stabilized with the 5-ethylphosphonate analog than with pyridoxal-P. ...

[page 550, last line, through page 551, first two lines]

B. Stridh et al., "The Effect of Pyrophosphate Analogues on Influenza Virus RNA Polymerase and Influenza Virus Multiplication," Archives of Virology 61:245-250 (1979) [copy attached to Supplemental IDS as Exhibit 20].

(1)

Analogues of pyrophosphate have been tested as inhibitors of influenza virus RNA polymerase activity in cell-free assays. ...

[page 245, abstract]

(2)

Several types of structures have been suggested as inhibitors of influenza virus multiplication (12). One type, which does not seem to have been systematically investigated, is analogues of pyrophosphate.

[page 245, 2nd full ¶]

(3)

Analogues of pyrophosphate were tested for inhibition of the virion associated influenza virus RNA polymerase under assay conditions essentially as described by BISHOP *et al.* (2).

[page 245, 3rd full ¶]

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In another scientific article published two years before this application was first filed, the term "sugar phosphate analogue" is disclosed and used. This article by Stoeckler et al. ("Human Erythrocyte Purine Nucleoside Phosphorylase Reaction with Sugar-Modified Nucleoside Substrates," Biochemistry 19:102-107 (1980); copy attached to Supplemental IDS as Exhibit 21] provides at least twelve (12) descriptions relating to the use of the term "sugar-phosphate analog." These twelve descriptions are listed below.

(1)

The kinetic parameters (K_m and V_{max}) of sugar-modified analogues of inosine and guanosine have been determined . . . The sugar phosphate analogue, 5-deoxyribose 1-phosphate, was synthesized from 5'-deoxyinosine with immobilized PNP, and its presence was verified . . . The adenosine versions of the 5'-modified analogues were also found to react with adenosine deaminase, albeit at 1% of V_{max} .

[page 102, abstract]

(2)

Further, the remarkably high activity (10-15 units/ml. of cells) of PNP in human erythrocytes may degrade various nucleoside analogues of chemotherapeutic potential in transit through the blood stream to the desired site of action.

[page 103, left column, 1st ¶]

(3)

The present report documents the activity of human erythrocytes PNP with a number of sugar-modified adenosine analogues by reaction with calf intestinal adenosine deaminase.

[page 103, 1st full ¶]

(4)

. . . Adenosine analogues were converted to their inosine counterparts by deamination . . .

[page 103, right column, 1st ¶]

(5)

The phosphorylation and synthesis of guanosine and its analogues were monitored directly at 258 nm . . .

[page 103, right column, 1st full ¶]

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(6)

Many of the inosine analogues examined were prepared from the respective adenosine analogues through deamination by reaction with calf intestinal adenosine deaminase. Since these adenosine analogues varied greatly in reactivity with ADA, it was necessary to adjust the ADA concentration and the reaction time to the particular adenosine analogue. In each case, formation of the respective inosine analogue was monitored spectrophotometrically by measuring the decrease in absorbancy at 265 nm. The adenosine analogues (1.0-5.0 nM) and 1-10 units of calf intestinal ADA were incubated in 2-mL reaction volumes for time periods necessary for complete conversion to inosine analogues, i.e., a few seconds to several hours.

[page 104, left column, 1st ¶]

(7)

Immobilized Enzymes for the Synthesis of 5-Deoxyribose 1-Phosphate
For use in the synthesis of inosine analogues from adenosine analogues and in the preparation of 5-deoxyribose 1-phosphate, PNP and adenosine deaminase were immobilized on agarose by a modification of published procedures (Pharmacia Fine Chemicals, 1976) . . .

[page 104, left column, 2nd ¶]

(8)

Results

Activity of Sugar-Modified Analogues with PNP.

Table I presents the kinetic parameters of the compounds studied . . .

[page 104, lower right column]

(9)

Substrate Activation

. . . As shown in Figure 2, similar substrate activation occurred at high concentrations of the sugar-modified analogues 5'-deoxy- and 2',5'-dideoxyinosine . . .

[page 105, left column, 1st full ¶]

(10)

Substrate Activity with Adenosine Deaminase The inosine analogues no. 3-11 of Table I were produced from the respective adenosine analogues, which were not active with PNP. When the adenosine analogues were incubated with calf intestinal adenosine deaminase, it was observed that those modified at C(5') were deaminated to completion but at extremely low rates . . .

[page 105, right column, 1st full ¶]

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(11)

Although there have been brief reports of the interactions of various sugar-modified nucleoside analogues with PNPs from several sources, there have been no detailed analyses of the kinetic parameters of this class of analogues with human erythrocytic PNP . . . Therefore, since many of the nucleoside analogues under consideration in this paper and elsewhere have potential as chemotherapeutic or immunosuppressive agents, it is important to appreciate that one may not be able to predict the reactivity of such analogues with human PNP on the basis of the studies performed with enzymes from other species.

[page 106, left column, last ¶, through right column, 1st ¶]

(12)

. . . A nucleoside analogue with good reactivity with deoxyguanosine kinase but low activity for PNP might form cytotoxic analogue nucleosides in T lymphocytes without prior degradation by PNP. Also, if potent inhibitors of PNP could be identified and coadministered with analogues of deoxyguanosine, e.g., β -D-2'-deoxythioguanosine, enhanced intracellular formation of analogue deoxynucleotides, e.g., deoxythioGTP, might be achieved. The most potent PNP inhibitor reported to date, the inosine analogue formycin B, has a relatively high K_i value (1×10^{-4} M) but is capable of inhibiting the phosphorolysis of 6-thioinosine in intact erythrocytes (Sheen et al., 1988) . . . Since large quantities of this enzyme are readily purified from human erythrocytes and high activity may be bound in a stable form, i.e. ~50 units/g of Sepharose, even sugar-modified nucleosides that display low V_{max} values with PNP may be converted to the respective pentose 1-phosphates through the use of prolonged incubation times. These novel pentose 1-phosphates may be used for the synthesis of new families of nucleosides through prolonged incubation with agarose-bound PNP and base analogues such as 6-thioguanine, 6-selenoguanine, and 8-azaguanine.

[page 106, left column, last ¶, through page 107, 1st seven lines]

That those persons in the art of nucleic acid technology, including sequencing, detection and chromosomal characterization, would appreciate and understand the meaning and the limits conveyed by the claim language (nucleotide analog which can be attached to or coupled to or incorporated into DNA or RNA) is also bolstered by the U.S. patent literature. A brief survey of this literature shows that numerous U.S. patents have been issued with claim language such as "nucleotide or nucleotide analog," or its equivalent "oligonucleotide or oligonucleotide analog." Listed below are sixteen (16) patents, together with reference to one or more claims reciting such language.

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<u>Inventor(s)</u>	<u>U.S. Pat. No.</u>	<u>Language/Claim Nos.</u>
Ryser et al.	4,847,240 (Exhibit 22 IDS)	claim 21 (drug nucleotide or nucleotide analog)
Schwartz et al.	5,212,059 (Exhibit 23 IDS)	claim 1 (said probes comprise a selective sequence of 10 to 100 nucleotides or nucleotide analogs)
Banker et al.	5,643,730 (Exhibit 24 IDS)	claim 11 (in the presence of one or more radiolabeled nucleotides or detectable nucleotide analogs)
Usman et al.	5,652,094 (Exhibit 25 IDS)	claim 3 (The enzymatic nucleic acid molecule . . . wherein W and Y together comprise at least one nucleotide or nucleotide analogue . . .)
Eigen et al.	5,807,677 (Exhibit 26 IDS)	claim 23 (A method . . . carried out using nucleotides or nucleotide analogs . . .)
Liu et al.	5,914,230 (Exhibit 27 IDS)	claims 10 & 34 (. . . each of said linking groups comprising from 0 to 40 nucleotides or nucleotide analogs)
Wright et al.	5,998,383 (Exhibit 28 IDS)	claims 1, 3, 5 & 6 (A synthetic antisense oligonucleotide comprising at least twelve nucleotides or nucleotide analogues)
Pagano et al.	5,242,906 (Exhibit 29 IDS)	claims 1-2 (An oligonucleotide or oligonucleotide analog consisting essentially of . . .)

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<u>Inventor(s)</u>	<u>U.S. Pat. No.</u>	<u>Language/Claim Nos.</u>
Ecker et al.	5,591,600 (Exhibit 30 IDS)	claims 1-7 (method . . . oligonucleotide or oligonucleotide analog . . .)
Anderson et al.	5,591,720 (Exhibit 31 IDS)	claim 1 (An oligonucleotide or oligonucleotide analog having . . .)
Cook et al.	5,614,617 (Exhibit 32 IDS)	claims 1-3 & 6-7 (oligonucleotide or oligonucleotide analog comprising . . .)
Baker	5,643,780 (Exhibit 33 IDS)	claims 21-23 & 25-27 (oligonucleotide or oligonucleotide analog)
Rahman et al.	5,665,710 (Exhibit 34 IDS)	claim 1 (method of encapsulating an oligodeoxynucleotide or an analog thereof) & claim 3 (. . . separated from oligodeoxynucleotide or oligodeoxynucleotide analog)
Ecker et al.	5,736,294 (Exhibit 35 IDS)	claims 1-6 (method . . . oligonucleotide or oligonucleotide analog)
Crooke et al.	5,811,232 (Exhibit 36 IDS)	claim 1 (An oligonucleotide or oligonucleotide analog) & claim 2 (method . . . oligonucleotide or oligonucleotide analog) & claim 3 (kit . . . oligonucleotide or oligonucleotide analog)
Ecker et al.	5,874,564 (Exhibit 37 IDS)	claims 1-15 (oligonucleotide or oligonucleotide analog)

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<u>Inventor(s)</u>	<u>U.S. Pat. No.</u>	<u>Language/Claim Nos.</u>
Martin et al.	5,891,468 (Exhibit 38 IDS)	claim 13 (composition . . . oligonucleotide or oligonucleotide analog)

The information contained in the preceding thirty pages and attached Exhibits 1-38 illustrate the state and knowledge of the art at the time this application was first filed in 1982. Those engaged in the field of nucleic acid technology, including processes for nucleic acid sequencing, nucleic acid detection and chromosomal characterization, would clearly have understood not only the meaning but the metes and bounds of the subject matter covered by the claim language "nucleotide analog which can be attached to, coupled to or incorporated into DNA or RNA."

Before addressing the rest of the issues at hand, Applicants would like to bring to the Examiner's attention three (3) U.S. patents and claims directed to nucleic acid detection and sequencing processes. In these patents, practically no limitation with respect to the nucleotidyl components -- either in the nucleic base or the sugar moiety -- were imposed or required. These patents are listed below together with an exemplary claim.

Smith et al., U.S. Patent No. 5,821,058, licensed to Applied Biosystems, Inc./Perkin-Elmer, issued October 13, 1988, based on a priority filing date of January 16, 1984 (or later depending upon the subject matter by various C-I-P applications [copy attached to this Amendment as Exhibit 1])

Claim 28 of ABI/Elmer Perkin's '058 Patent recites:

A method of determining the sequence of a polynucleotide which comprises:

- providing polynucleotide fragments tagged with chromophores or fluorophores, wherein the chromophores or fluorophores are distinguishable from others by their spectral characteristics;
- resolving the polynucleotide fragments by electrophoresis; and
- detecting the resolved fragments by means of the chromophore or fluorophores, and thereby determining the sequence based on the polynucleotide fragments detected.

Enz-5(D8)(C2)